Society for Theriogenology

Veterinarians Dedicated to Animal Reproduction

Clinical Theriogenology

Official Journal of
The Society for Theriogenology
The American College of Theriogenologists

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and
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Mission Statement

The purpose of *Clinical Theriogenology* is to publish in a timely manner peer-reviewed information relevant to the clinical practice of theriogenology for veterinary practitioners, academic clinicians, and veterinary students. The journal will be the means by which the Society for Theriogenology (SFT) publishes the proceedings of its Annual Conference and Symposia.

Scope of the Journal

Clinical Theriogenology will be broad in scope and manuscripts published will be in the following categories:

- Research reports
- Reviews of current literature
- Clinical reports
- Innovative techniques
- Book reviews
- Letters to the editor
- Editorial opinion
- News from the Society for Theriogenology and the American College of Theriogenologists

Publication Schedule

The regular issues will be published quarterly. On occasion, the Editorial Board will consider issuing a Festschrift to honor eminent theriogenologists.

Manuscript Preparation

Manuscripts are accepted for consideration with the understanding that they have not been published elsewhere (except in the form of a brief abstract) and are not simultaneously under review by another journal. The manuscript must be in English (American spellings), and follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org). The following guidelines are applicable:

- Manuscripts should be submitted to the editorial office as an e-mail attachment or on a CD compatible with Microsoft Word. The disk should be accompanied by a hard copy of the manuscript. Submit manuscripts to:

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- All pages are to be double-spaced
- Font: Times New Roman; size 12
- Left-justified
- 1" margins at the top, bottom, and sides of each page
- All pages (including the title page) are to be numbered consecutively

- All lines of the manuscript should be numbered consecutively

The general format for scientific manuscripts is as follows:

- Title page: Contains the title of the paper and the first and last names of each of the authors; middle initials are optional. Specify the corresponding author and her/his contact information (mailing address, telephone and fax numbers, email address). Do not list academic degrees or specialty board certification. State the sources of funding (if any) and any meetings at which the data were presented.
- Abstract and keywords: The abstract should capture the essence of the paper and should be limited to 250 words or fewer. The term "Keywords" is typed in bold font followed by a colon followed by up to six key words separated by commas.
- Text: Begin the text on a separate page and divide it into the traditional sections of a scientific paper, viz. introduction, materials and methods, results, discussion, and conclusion.
- References: Only the most pertinent papers should be cited. References should be cited in consecutive order when first mentioned in the text, designated by a superscript number placed after all punctuation marks. The Vancouver style of citation is to be used with the exception that only the first three authors of multi-authored papers are listed; if there are four or more authors, list the first three, followed by et al. For examples, please consult: http://www.nlm.nih.gov/bsd/uniform_requirements.html Titles of journals are to be abbreviated in the style of Index Medicus. For assistance in locating the proper abbreviation for a scientific journal, see the following websites: http://home.ncifcrf.gov/research/bja - Biological Journals and Abbreviations. PubMed also has journal abbreviations available at http://www.ncbi.nlm.nih.gov/sites/entrez?db=journals Type the name of the journal into the search box and select "go". The next screen shows information about the journal including the proper abbreviation. One word titles are not abbreviated. Unpublished observations and personal communications should not be used as references. Articles listed as "in press" must have been accepted for publication. Authors are responsible for insuring the accuracy of all citations listed; citations must be verified against the original publications.

  Examples:
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  Odde KG: A review of synchronization of estrus in postpartum cattle. J Anim Sci 1990;68:817-830
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  Martinez MF, Adams GP, Kastelic JP, et al: Induction of follicular wave emergence for estrus synchronization and artificial insemination in heifers. Theriogenology 2000;54:757-769.
  Book (personal author)
  Johnson SD, Kustritz MVR, Olson PNS: Canine and feline theriogenology. Philadelphia: Saunders; 2001. p. 7.
  Book (edited, multi-author)
  Woods GL, Hallowell AL: Management of twin embryos and twin fetuses in the mare. In: McKinnon AO, Voss JL, editors. Equine reproduction. Philadelphia: Lea and Febiger; 1993. p. 532.
  Proceedings
  Kenny RM, Bergman RV, Cooper WL, et al: Minimal contamination techniques for breeding mares: techniques and preliminary findings. Proc Annu Conv Am Assoc Equine Pract 1975; p. 327-336.

- Units of measurement: Measurements should be listed in Système Internationale (SI) units with non-SI units listed in parentheses if they are needed for clarification.

- Abbreviations: Abbreviations other than standard units of measurement should not be used excessively but if necessary, the abbreviation must be defined at first use in the manuscript. Do not use abbreviations in the title or in the abstract. Abbreviations are used exclusively subsequent to being defined except at the beginning of a sentence.
- Tables: Data can be summarized in well-designed tables prepared with the table function of Microsoft Word. Each table should be on a separate page with the legend typed above the table. Tables are numbered consecutively as they appear in the text.
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- Sources and manufacturers: Only generic names of drugs, chemicals, test kits, and equipment should be used in the text followed in parentheses by the tradename, supplier's name, and supplier's address (city, state [country if not in the United States]). For example: The cow was treated with 100 mcg gonadorelin hydrochloride im (Factrel™, Fort Dodge Animal Health, Ft. Dodge, IA).
- Manuscripts are initially reviewed by the editor and those that meet the requirements for publication are submitted to at least two reviewers. Authors are invited to suggest potential reviewers in their initial submission. Manuscripts are prepared for publication in the order in which they pass the peer review process

Outline for Case Reports and Case Series

Title of Case
Authors of case. Please indicate corresponding author by * (after the author's name)
Summary. Up to 150 words summarizing the case presentation and outcome
Background. Why is this case important?
Case Presentation. Presenting features, pertinent medical history, herd history (if applicable)
Differential Diagnosis. (if relevant)
Treatment.
Outcome .
Discussion. Include a brief review of similar published cases; how many other similar cases have been reported?
Learning points. Three to five bullet points
References. Vancouver style
Figure/photo captions. (if any)

Instructions to Authors are subject to revision to meet the technical standards imposed by the printer and are to be reviewed annually and revised if necessary by the Editorial Board.

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Proceedings of the Annual Conference of the Society for Theriogenology

August 31 to September 5, 2010

Seattle, Washington, USA

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Camelids

Manuscripts from the post-conference symposia (Reproductive Pathology Symposium, Theriogenology Educators' Forum, and Canine Breeders' Symposium) and conference manuscripts not received in time to be included in the Proceedings issue will be published in subsequent issues of *Clincal Theriogenology*.

2010 Bartlett Address

Theriogenology: gratitude, recollections, thoughts and opinions

Robert L. Carson, Jr.
College of Veterinary Medicine, Auburn University, AL

I want to thank the selection committee for honoring me with this prestigious award. To be the recipient of an award in tribute to Dr. Bartlett is an extreme honor and very humbling. Frankly, there are not words to describe the honor bestowed on me nor my feelings and emotions. After reviewing the list of previous recipients the experience has become even more humbling. There is an expression used in my part of the world "walking in high cotton". By joining this elite group of individuals who I have admired and respected for years I am certainly "walking in high cotton". I can assure you that there are many other individuals more deserving of this recognition than myself, but certainly none more appreciative.

The only down side to this recognition is the conformation of passage into senior citizenship. Many people have mentored me and contributed to the development of my career and I will refer to them during this presentation. Several of them I will mention by name, but there are too many to name all of them and express my gratitude.

Most of all I want to thank my wife Karen for her love, support and tolerance throughout the thirty six years of our marriage. I also want to thank my children Jennie, Kelly and Trace for the same traits as their Mom love, support and tolerance. A wise old man once told me that children are like your dog or your horse. Sometimes you are so proud of them you could bust and there are other times you don't want to claim them. I have been able to be proud many more times than not claiming them.

When these two organizations established their beginnings in 1954 I was a six year old farm boy in the hills of East Tennessee destined to become a Theriogenologist. Little did I know that my path would someday cross that of some of those founders. I was the son of a farmer and an elementary school teacher. I want to thank my parents and grandparents for a wonderful childhood. Growing up on that farm molded me for the rest of my life. It was a diversified operation with crops of tobacco, wheat, oats, corn, soybeans and a wide variety of fruits and vegetables. Our livestock consisted of beef and dairy cattle, horses and mules, sheep, goats, hogs, hunting dogs, barn and house cats and a variety of poultry. Not many things came from the grocery store. By nine years of age I knew I wanted to become a veterinarian. I knew the gestation length and number of offspring of all the various farm animals we had. I knew the orifice those offspring came out of and to the horror and embarrassment of my mother and grandmother I knew how they got in there. I inherited my love of animals and curiosity from my Dad and my desire and ability to teach from my Mom and I thank them for those traits. I also want to thank them for their financial support and sacrifices which allowed my sister and me to complete our educations with very little debt.

I owe a debt of gratitude to all my undergraduate professors. I am especially grateful to the faculty of Auburn University College of Veterinary Medicine between 1969 and 1973 for their efforts. I had no idea at the time that I would eventually become one of them.

I graduated from Auburn in 1973 and went into a mixed practice in North Carolina working for Dr. Lewis Puckett. I will forever be grateful for his patience and all he taught me. Practice was good for me. I learned a lot and even met my wife during that time. However; after a while I realized that our practice was not fulfilling my greatest professional interest and desire: Theriogenology.

In those days most of the advanced technology, modern equipment, science and research seemed to be associated with veterinary colleges. (Due to budgetary issues that is not necessarily true today.) So my next step to quench my thirst for Theriogenology was to do a residency.

In 1975 I became a Theriogenology resident at the University of Georgia. I had some great mentors and friends there: Drs. John McCormick, John Williams, Don Witherspoon, Al Caudle and Steve Van Camp. I thank them for their efforts and friendship.

In 1978 I became an assistant professor in the Department of Large Animal Surgery and Medicine at Auburn University where after thirty-two years I am still employed. In 1978 this was a dream job. I was now a colleague of the Large Animal Clinic faculty who had taught me as a veterinary student. A faculty I still have a tremendous admiration and respect for. I will always be grateful for the support and mentorship of Drs. Bob Hudson, Tom Vaughn, Don Walker, John Winkler, Jay Humburg, Agee Wiggins, Ram Purohit, and Tom Powe. To be able to work closely with those individuals at the beginning of my academic career was an awesome experience. I cannot speak of Auburn without recognizing two of my colleagues of close to thirty years and thanking them for their friendship, cooperation and support: Drs. Dwight Wolfe and Gatz Riddell.

Some of you will remember the meeting that did not happen: Mobile Alabama 1979. Candidates for the oral portion of the Diplomate examination were tested on Tuesday September 12 while Hurricane Fredrick picked up speed in Mobile Bay. I was one of those candidates. After completing my orals I headed for the safety of higher ground and returned 225 miles inland to Auburn. The candidates who chose to weather the storm found out their results shortly before the storm made land fall and then were exposed to one of the more frightening experiences of their lives. Dr. Bob Hudson had chosen to weather the storm in Mobile. Back at Auburn on Wednesday one message had gotten through from him that everybody was alright, but the meeting was canceled. No word regarding my success or failure. I was relieved that everybody had weathered the storm without any serious consequences, but was concerned about my results. I imagined that Dr. Hudson wanted to give me the bad news in person, and decided I would take a week off then start studying again. I will always remember three days after the examination Friday morning September 15 an extremely tired, sleep deprived and worn out Dr. Bob Hudson congratulated me in the office we shared on becoming a Diplomate of the American College of Theriogenologists; he wanted to give me the good news personally.

I would like to express my sincere gratitude and thanks to the twenty two plus Theriogenology residents and graduate students that I have had the privilege to either direct or assist in the direction of their training. I want to thank them for their diligence, intellect, hard work and enthusiasm. Often I learned more from them than they did from me. They have gone on to have very successful careers of their own. One of them I would like to especially recognize who never reached his potential Dr. Allen Heath who tragically lost his life in 2003.

Another group of Auburn people I want to thank and recognize are the members of the Food Animal Section including faculty, residents and technicians both past and present. I have had the privilege of being their Section Chief for over fifteen years. They are without question some of the hardest working, most cooperative, talented and intelligent individuals one could ever know and work with.

I appreciate the confidence and trust of clients and referring veterinarians. It is always flattering when they seek your advice and opinion and trust your clinical capabilities.

I cannot have spent over thirty-five years in academia without recognizing one of the primary reasons I chose this career path: the students. Combining Georgia and Auburn I have had the good fortune to teach over 3200 veterinary students. On clinical rotations I have been able to get to know them quite well, and in many cases life-long friendships have been created. It is impossible to remember all their names and faces, but I applaud them for their enthusiasm, efforts and endurance of reaching their dream of becoming veterinarians.

Theriogenology as a recognized specialty is relatively young when compared to some other specialties; however, as a practiced discipline it is ancient. Theriogenology began when man first decided to selectively mate animals. Early man investigated facts we just assume have been known forever simple but yet complex facts. An example is the gestation length of the various species a simple but time consuming controlled study. I consider myself a second generation Theriogenologist benefiting from a wealth of knowledge generated by the first generation; those individuals who started these two organizations. Individuals like Dr. Bartlett and Dr. Roberts, and other individuals on the list of previous recipients of this prestigious award. Scientists who were frequently armed with only their physical senses, ability for physical diagnosis, a desire for knowledge, the tenacity and perseverance to learn and the willingness to share their discoveries. We owe those founders and forbearers a tremendous debt.

As a second generation Theriogenologist I have seen many changes and advances. Procedures, products and technologies that are commonplace today seemed to have developed over night. PGF2a and GnRH used daily by many of us were only identified some thirty odd years ago. Frozen semen was pretty much limited to the bovine species. Sexed semen was merely a desirable idea. Embryo transfer also was limited primarily to the bovine species. Embryo collection was accomplished by surgically flushing the uterine tube. Transfers were also surgical and success was limited to only fresh embryos. Frozen embryos, follicular aspiration, in vitro fertilization and fetal sexing were research ideas that would materialize into common practices. The thought of cloning was introduced to the general public in a 1978 movie starring Gregory Peck titled the "Boys from Brazil". Manuscripts, thesis, and dissertations were done on electric type writers. A change to one sentence frequently resulted in retyping the entire document. Literature searches were done manually by wading through volumes of Index Medicus and Index Veterinarius. Peer reviews were dependent on the postal service and were quite time-consuming. Hormonal assays were expensive, labor intense, time-consuming and frequently had a wide margin of error. The first ultrasound machines had a screen about one-half the size of a postcard and an image that was likened to that of a black

and white television with poor rabbit ear reception. Computers were large cumbersome devices occupying rooms and buildings not desks and laps. Yes, we have come a long way, and the third, fourth and beyond generations of Theriogenologists will go even farther.

The science of Theriogenology covers a diverse population of species. Career choices in Theriogenology are as diverse as the species we study. Broad categories of careers include but are certainly not limited to private practice, research laboratories, industry and academia. Each of these career pathways is dependent on the others and all are essential to the practice and advancement of Theriogenology.

The private sector is where many new ideas and procedures originate. The private sector is also a place where both common and new problems are first recognized. It is also the ultimate proving ground for new products, technology, and procedures. The research laboratory is the arena for new discoveries and the advancement of basic knowledge. Industry takes new discoveries and new ideas and develops them into products and technologies, and makes those available to all. Academia frequently requires research and development as well, but is saddled with the responsibility of incorporating basic knowledge and recent advancements into the minds of our future colleagues. None of these career choices is more important than any of the others. That being said each deserves respect, appreciation and admiration from the others. Regardless of our career path we are dependent on the success and knowledge of those who chose a different direction. As you attend the scientific sessions over the next few days you will witness representation from all those career categories, and I hope you will come away with an appreciation of the dependency we have on one another. I encourage you to attend a session regarding a species other than that of your primary interest. You will learn something new and will gain an appreciation and respect for those in a different species arena.

I have discussed a little bit about the past of Theriogenology now I would like to give you my thoughts and opinions on the present and future. Think about this for a moment, if Theriogenology fails so does all veterinary medicine. Our discipline is responsible for the control, continuation and conservation of the animal population. We have now entered an era of what can best be described as a scientific explosion; an explosion consisting of once unimaginable advancements of knowledge, technology, information and capabilities. The question is what are we going to do with this explosion? We are going to have more tools available to us than ever before. The benefits of this scientific explosion; however, are not just gifts without strings attached. These benefits come with responsibilities and challenges.

We cannot afford to be satisfied and complacent with the status quo, therefore we all need to be participants in the advancement of our discipline. We must remember lessons learned from the past. Lessons like the ability to use our physical senses and being able to perform a good physical examination, and remembering to examine the whole animal not just the reproductive system. We must remember population medicine to examine the entire group not just the ones that are obviously affected. We must have respect and compassion for the animals entrusted in our care. Owner communication, respect, honesty and compassion will always be essential. Moral and ethical responsibilities, challenges and decisions will increase with this scientific explosion. We must never lose sight of practicality and always have the good sense to recognize our limitations and be able to admit when nothing else can be done. Just like in the past we will make mistakes, have failures and successes. We must learn from our mistakes and failures because if we do not we are likely to repeat them. After mistakes and failures we will need to have the tenacity and perseverance to keep trying. We should enjoy our successes but must not over indulge in self-worth. We must be willing to share developments and advances for the benefit of all. We must not let egos get in the way of scientific advancement. Professional behavior and respect for colleagues must remain essential to our character. Development and advancement of Theriogenology will only continue to grow and the possibilities of the future are beyond imagination.

I will admit struggling with a summary and closing of this presentation especially when looking toward the future, but there it was hanging on the wall in my office. You will recognize these words which are relevant to the past, present and future.

> *Being admitted to the profession of veterinary medicine, I solemnly swear to use my scientific knowledge and skills for the benefit of society through the protection of animal health, the relief of animal suffering, the conservation of livestock resources, the promotion of public health and the advancement of medical knowledge.*

I will practice my profession conscientiously, with dignity and in keeping with the principles of veterinary medical ethics.

I accept as a lifelong obligation the continual improvement of my professional knowledge and competence.

Thank you for this prestigious honor and for listening to me.

Reproductive applications for alternative/complementary care in veterinary medicine; acupuncture, chiropractic, manual therapy; treatment, and diagnosis; a neuroanatomical review

Tim N. Holt
Veterinary Medical Center, Colorado State University, Ft. Collins, CO

In this presentation, the author will discuss the anatomy and neurophysiology of manual therapy and acupuncture as it relates not only to pain modulation but also to reproductive performance. The mechanisms of action are multifactoral and interactive. This discussion will explain not only why the author chooses to do acupuncture and manual therapy for the client, but also how these therapies may affect the animal through neurological modulation. The history of acupuncture and its introduction into the western world will be briefly reviewed. Also reviewed will be the nervous system and how it can be manipulated through acupuncture, acupressure, aquapuncture, electrical stimulation, and moxa. Pain modulation and control of pain through acupuncture and trigger point therapy and its relationship to reproductive success will be discussed. The audience will learn specific points to address common problems seen in small animal medicine as well as large animals including equine, bovine and camelids and how acupuncture can be used as a complementary alternative modality. Some time will be spent on myofascial work and trigger point therapy and how it may mask or manifest itself in lameness, musculoskeletal pain, and reproductive failure. Participants will be given point combinations that involve both TCM and neuroanatomical acupuncture dealing with pain, musculoskeletal disease, and reproductive emergencies as well as reproductive failure. The mechanism by which these complementary modalities have the potential to inhibit pain and how they can be used in different situations will be discussed, as well as discuss when they may not be appropriate first line treatments. Also discussed will be how these modalities can address reproductive issues, loss of performance, musculoskeletal pain and stiffness, as well as some of the metabolic situations where acupuncture and manual therapy may be indicated as complementary care in dealing with post-operative ileus, laminitis in large animal medicine, and pain as well as endocrine diseases such as Cushing's and renal disease in small animal medicine. The focus will be on the practical use of complementary medicine in a private practice setting. Cases will be discussed including the treatment that was used as well as the outcome. The uses of acupuncture will be presented and the success seen in each case; what are the most common situations it is used for and how cases are approached for best results. The pros and cons of acupuncture and manual therapy will be presented as well as the possible side effects. Contraindications to acupuncture and manual therapy and potential complications of each will be discussed. Other topics will include acupuncture and manual therapy for the cancer patient after chemotherapy and their uses in the pregnant animal and acupuncture for endocrine disorders. Participants will have the opportunity to see materials available for their use as well as electrical stimulation and its use. The types of acupuncture points and selection of points will be outlined according to clinical signs the clinician is presented with including the type of points and how they are used as well as the anatomical location of each.

This presentation will be a basic introduction to acupuncture and manual therapy, designed to give the clinician an understanding of the mechanics involved in stimulation of the nervous system through neuro-modulation. Scientific articles will be available or cited for future use. The presentation is not designed to go into great depth or certify the participant as an acupuncturist or manual therapist but to present the basic foundation of acupuncture and alternative therapies allowing practitioners to better communicate with their clients.

Acupuncture has been evolving over the last 3000 years. This type of complementary care is being utilized more every day by equine practitioners, small animal surgeons and internists, and those involved solely in pain control. The demand from clients for the use and utilizations of alternative medical therapies continues to rise. Acupuncture specifically is used in numerous situations. Its popularity continues to rise which is visible in the numerous lecture topics at many veterinary conferences. Acupuncture is the art of inserting a needle into specific regions of the body that are identified as acupuncture points that are anatomically identifiable. Acupuncture points, due to their increased vascular supply, presence of numerous mast cells, neuro-bundles and decreased electrical resistance stimulate the central nervous system (CNS) in numerous interacting mechanisms. Stimulation of these points then modulates the CNS through local effects, spinal stimulation as well as central and hormonal effects.

Manual therapy is a term that is used to include chiropractic, osteopathy, massage and physical therapy. It is a means of manipulating and stimulating receptors in the body. Most joint injuries are secondary to a failure of the soft tissue of the supporting structures. These supporting structures are most commonly the muscles surrounding a joint. For optimum muscle health the muscle must have motion through active neuro stimulation, glucose and oxygen. Manual therapy is a means of maintaining good joint and muscle health through stimulation of the mechanoreceptors as well as muscle spindle cells. Efficiency of a joint is dependent on the efficiency of the muscles that support it. The health/efficiency of the muscle is dependent on the frequency of firing of the motor neuron

supply to that muscle. The frequency of firing of the motor neurons is dependent on the summation of neural influences in a multi-modal system. This summation of neural influences is then dependent on the spinal cord reflexes, brain, and integration of sensory input from the environment. Receptors that both acupuncture and manual therapy target are nociceptors (A-delta, C-Fibers). A-delta fibers are very small, slow, slightly myelinated nerves that carry sharp pain. A-delta fibers are responsible for the "de qi", a term used in Chinese medicine that means muscle, myofascial contraction or fasciculation upon stimulation via a needle or manipulation. Mechanoreceptors are those receptors that transduce somatic sensation of touch, tactile, joint position and vibratory sensation to the CNS. These sensations are carried to the dorsal horn of the spinal column through large diameter myelinated fibers 1A and 1B very fast fibers, thus the gate theory of pain control. The gate theory states that by eliciting stimulation of very fast myelinated 1A and 1B neurons as well as the alpha motor neuron that their arrival at the spinal segment prior to the slower harder to stimulate nociceptors, A delta and C-fibers, may dampen perception of pain. In summary, it is the summation of all excitatory and inhibitory influences on the motor neuron that will determine its frequency of firing and thus its strength.

Acupuncture and manual therapy are complementary modalities to maintain health and better the quality of life when western medicine falls short. These types of therapies can help relieve pain, restore loss of performance including reproductive performance, or any other medical situation which does not respond to a western approach. After a western medical diagnosis is made, or attempts have been made to diagnose a problem, these methods can be used to amplify or assist in what is being done medically. In manual therapy the goal is to stimulate receptors to regain motion in an affected area thus returning it to a balanced state. Kevin Haussler, DC, DVM states, "The goal of chiropractics or manual therapy is to optimize health through the body's inherent healing ability, to offer homeostasis as affected by and integrated through the nervous system." Dominque Giniaux states that osteopathy is not a set of manipulations, it is a particular approach to the equilibrium of a living organism and its pathology, and Andrew Still, MD, one of the first MD's to begin osteopathic medicine based his therapies on "structure governs function". It is important in all therapeutic techniques regardless of which modality is being utilized to return the animal to its most balanced state allowing healing of all structures through the CNS, to balance the yin and yang.

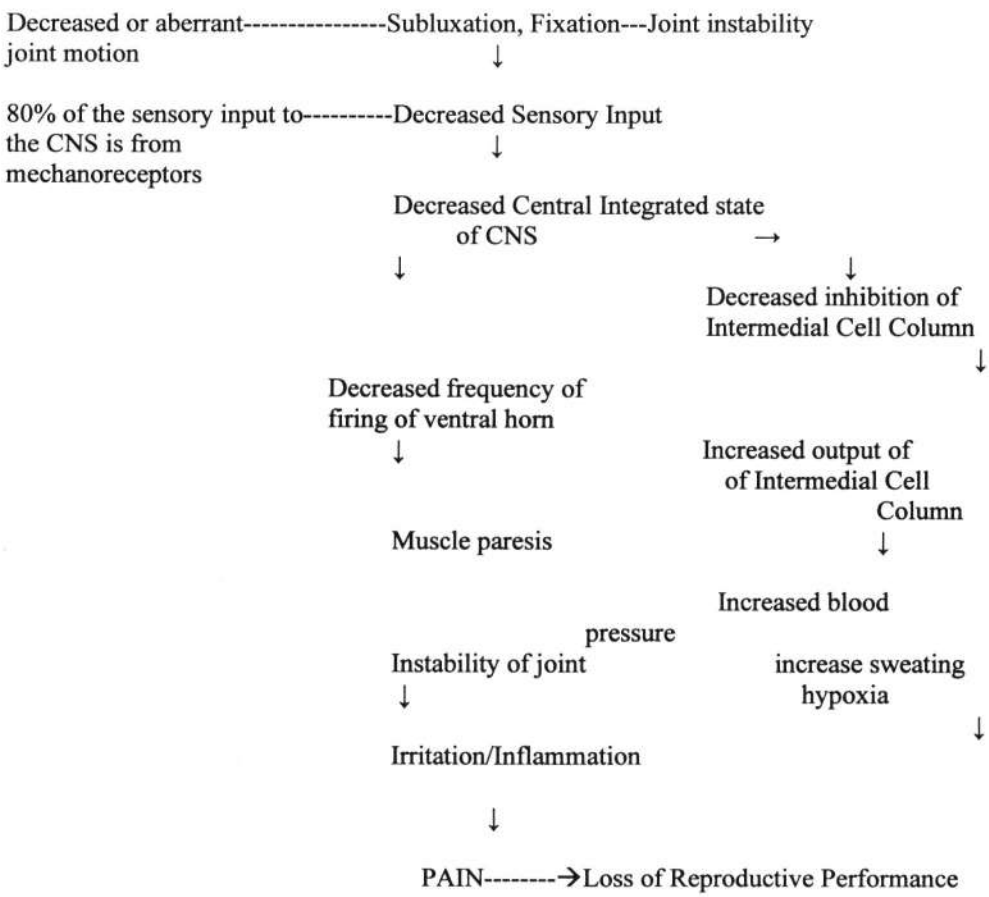
Response to acupuncture and manual therapy can vary between each case and is reliant on individual differences. Often the outcome is dependent on the severity of the pathology as well as the chronicity. Most commonly the response is seen; if the musculoskeletal system is involved, that the following day is slightly more painful or stiff. It often takes the CNS over 24 hours to adjust to the manipulations or needling. This delayed response is common and is followed by steady improvement over time. It is not advised to do manual therapy daily or in most cases weekly. Often rest and patience between treatments is required to allow the body to heal once it has been treated. Treatment may be no more than relieving a trigger point or regaining motion in a joint that has been restricted for a long period of time. Even what may seem like very little takes a complicated neuronal afferentation to achieve wellness. In acupuncture the most common means of treatment involves two to three treatments over the first two weeks then as needed monthly to once every six weeks. These examples of course are dealing with the situation in which there is pain often secondary to lack of normal motion of a joint, which may result in not only joint pain but muscle pain as well. Each animal is different as is each situation they may be presenting for. Immediate results are more commonly seen when treating post-operative ileus or gastrointestinal problems that are secondary to other metabolic conditions being treated with conventional therapies. A good prognosis is most often the case if the animal makes a significant change in a musculoskeletal disorder within a week to month. If an endocrinology or organ dysfunction is being treated it may take months to return the body to normal. Not unlike western medicine, these modalities have their limits and should be used remembering this. These therapies cannot heal a fracture but they can help in controlling pain associated with the fracture or help in revascularization to the traumatic area. These modalities must be explained to the clients in terms they can understand and used with this in mind.

Acupuncture and manual therapy can be used for many situations in a private practice. Some of the most common utilizations of these modalities include: Musculoskeletal issues, subluxation (for the most part good success): degenerative joint disease, intervertebral disk disease (no chiropractic technique at site of lesion), nerve injury/paralysis, back pain, muscle pain, trigger point therapy, lameness, laminitis, and tendon injuries. Gastrointestinal: (variable results, most good depending on etiology) diarrhea, ileus, non-surgical colic, chronic colic and inflammatory bowel. One of the most upcoming uses of complementary therapies are their use in reproductive performance.

Other areas include but are not limited to skin disease, atopy or allergies, hives, wound healing, indurated wounds, reproductive issues including irregular heat cycles, uterine fluid retention, ovulation, cystic corpora lutea, poor libido, poor semen quality, urinary disease, renal disease and some respiratory issues.

In summary, this discussion will include the effects of acupuncture and manual therapy as well as how to implement these modalities into a practice. It is just as important to know when to choose one of these modalities as it is to know how use it. Patients and clients should be selected with care. Always have a good western medical work up and use these to amplify or add to your treatment plan. Practitioners will learn the art of palpation again and observe how this can lead them to regions on the animal that are not functioning normally thus causing pain, disease, or metabolic issues. The basic reasoning behind these modalities of treatment are to stimulate the CNS through neurological receptors and help the body return to a balanced state. This can be done through acupuncture points and needles or through manual therapy, osteopathy, and chiropractic techniques that will be described later. Clients will benefit from the availability of these as a part of a treatment plan. Alternative treatment options can be offered to clients, all of which if done correctly and used correctly have minimal side effects and can be used simultaneously with most western medical therapies as well as drugs. Remember veterinarians that utilize these techniques are virtually stimulators of receptors that allow the body's own abilities to heal through the CNS.

Neurological Model for Pain through Manual Therapy (modified from DeStefano, 2002)



Suggested reading

1. Clayton HM, Flood PF, Rosenstein DS: Clinical anatomy of the horse. St. Louis: Mosby; 2005.
2. Denois J-M, Pailloux J-P: Physical therapy and massage for the horse. 2 ed. North Pomfret (VT): Trafalgar Square Books; 2001.
3. Done SA, Goody PC, Stickland NC, et al: Color atlas of veterinary anatomy: volume 3, the dog and cat. 2nd ed. St. Louis: Mosby; 2009.
4. Filshie J, White A: Medical acupuncture: a western scientific approach. London: Churchill Livingstone; 1998.
5. Fleming P: Equine atlas of acupuncture loci. Dade City (FL): Florida Equine Acupuncture Center; 2000.
6. Gatterman M: Foundations of chiropractic: subluxation. St. Louis: Mosby; 1995.
7. Getty R: Sisson and Grossman's anatomy of domestic animals. 5th ed. Philadelphia: Saunders; 1975.
8. Giniaux D: What horses have told me: an essay on equine osteopathy. Cleveland heights (OH): Xenopnon Press; 1996.
9. Haussler K, editor: Back problems. Vet Clin North Am Equine Pract 1999. Vol 15, No 1.
10. Kendall DE: Dao of Chinese medicine. New York: Oxford University Press USA; 2002.

11. Maciocia G: Foundations of Chinese medicine: a comprehensive text for acupuncturists and herbalists. 2nd ed. London: Churchill Livingstone; 2005.
12. Schoen AM: Veterinary acupuncture: ancient art to modern medicine. St. Louis: Mosby; 2001.
13. Schwartz C: Four paws, five directions: a guide to Chinese medicine for cats and dogs. Berkeley (CA): Celestial Arts Publishing; 1996.
14. Smith GW, Davis JL, Tell LA, et al: Extralabel use of nonsteroidal anti-inflammatory drugs in cattle. J Am Vet Med Assoc 2008; 232:697-701.

Emerging diagnostic approaches for evaluation of fetal and pregnancy well-being in the mare

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Abstract

Placental insufficiency is regarded as the primary factor contributing to late-term abortion, pre-term delivery and perinatal death of foals. Often when problems associated with late-term pregnancy in the horse manifest the condition is well advanced and therapeutic intervention may not be effective in rescuing the pregnancy. If a compromised pregnancy could be identified early, the pregnancy might be sustained through appropriate medical intervention. The challenge is determining the most effective diagnostic approach that will correctly assess the status of the pregnancy and the uterine environment. It is highly unlikely that any one test will serve this purpose; rather a combination of diagnostic approaches may be necessary to accurately assess the well-being of the pregnancy and the fetus. As in human pregnancy, the most common cause of later term pregnancy loss and pre-term delivery is associated with some form of placental insult or deficiency which places the pregnancy at "high risk". Thus, this review will focus on current diagnostic approaches (i.e., endocrine markers, transabdominal/transrectal ultrasound, Doppler ultrasound) utilized in equine medicine and also describe emerging techniques in human medicine (i.e, protein biomarkers in amniotic or cervico-vaginal fluids, bioluminescence imaging technology) that could be applied to the mare to identify "at risk" pregnancies.

Keywords: Emerging diagnostics, at risk pregnancies, equine

Introduction

It is well documented that pre-term deliveries in women are a leading cause of neonatal mortality and morbidity and increase the likelihood of other pathologies occurring in the surviving neonate.[1] Offspring of preterm deliveries that do survive display delayed developmental progress and also have a greater chance of contracting disease and developing other chronic disorders in early life.[1-3] Additionally, it is known that intra-amniotic infections are implicated as one of the causative factors of preterm birth.[4,5] Similarly, pregnancy loss during late gestation and death of foals weakened by abnormal periparturient events constitute a large percentage of fetal and neonatal mortality in horses.[6] Placental infection due to opportunistic pathogens (i.e., *Streptococcus equi* subspecies *zooepidemicus* or *Escherichia coli*) is the single most common cause of abortion, stillbirth and premature delivery in horses.[7-9] Based on pathology records of horses, Giles and colleagues reported that 60% of fetal abortions, stillbirths and foals that died within 24 h of birth were associated with placental insufficiency, of which a third of the abortions and fetal deaths were associated with placental infections.[7] Moreover, mares most commonly afflicted tend to be pluriparous.[10] Due to the expense of breeding contracts and the long gestation of the mare, late-term fetal death represents a major financial loss and time investment for the breeder. Early identification of placental insufficiency would make it possible to sustain the pregnancy through medical intervention. For example, when placental function is compromised in mares suffering from fescue toxicosis, timely drug therapy improves pregnancy outcome.[11-13] Likewise, if diagnosed early, placentitis can be treated with systemic antibiotics, progestins (progesterone, 17α- hydroxyprogesterone, 5α-pregnanes), non-steroidal anti-inflammatory drugs or corticosteroids in an attempt to hasten fetal organ maturation.[14,15] However, despite this knowledge, a full understanding of the underlying pathophysiology of preterm birth is still lacking, and diagnostic tools, which can accurately and reliably identify "at risk" pregnancies, do not readily exist.

This review will focus on current diagnostic approaches (i.e., endocrine markers, trans-abdominal/transrectal ultrasound, Doppler ultrasound) utilized in equine medicine and also describe emerging techniques in human medicine (i.e, protein biomarkers in amniotic or cervico-vaginal fluids, bioluminescence imaging technology) that could be applied to the mare to identify "at risk" pregnancies.

Endocrine markers

Late gestation abortions and pre-term deliveries in the mare are commonly associated with disruption of normal endocrine function due to placental insult from infectious pathogens, and/or pathological changes to the feto-placental unit or due to serious maternal illness (i.e., colic) or disease. LeBlanc has classified three groups of hormones involved in pregnancy based on their physiological roles: for example those involved in uterine quiescence (i.e., progestins), those that stimulate uterine contractions (i.e., prostaglandins, oxytocin) and those

involved in fetal maturation (i.e., glucocorticoids).[10] One may add relaxin, a small polypeptide hormone that is found during pregnancy in many species including the equine.[16] To this end, serum-based hormone assays have been used in the past to determine pregnancy and fetal viability in the horse[17,18] and are likely to continue to be used to monitor pregnancy well-being in the equine.

Progestins

In most mammalian species, progesterone (P4) is essential for maintenance of pregnancy and may be produced by the ovary as in the sow and/or the placenta as in the mare. This C-19 steroid controls the duration of pregnancy by maintaining myometrial quiescence.[19,20] The horse is unique among domestic species in that at about Day 35 of gestation a discrete thickening of the trophoblast develops on the outer surface of the chorion to form what is described as the chorionic girdle and later form endocrine-secreting structures known as the endometrial cups.[21] From approximately Day 40 through to Day 70 of gestation these structures secrete equine chorionic gonadotropin (eCG) that has luteinizing hormone-like activity which ovulates successive waves of follicles stimulated to develop on the ovary under the steady release of pituitary derived follicle stimulating hormone (FSH) during the first half of pregnancy.[21] These secondary corpora lutea secrete progesterone and persist until such time that the chorioallantoic placenta is well enough established to take over completely the supply of sufficient progesterone to maintain the pregnancy until term (see review).[21] However, during the second half of pregnancy P4 is rapidly metabolized into several related progestagens (pregnananes and pregnenes) as previously reported,[22,23] but little is known about the biological activity of these P4 metabolites. Thus, from Day 40 to Day 100 of gestation, progesterone could be used as a marker of pregnancy well-being. However, by Day 85 of gestation, the feto-placental unit begins to synthesize large quantities of steroid hormones utilizing fetal C-19 precursors secreted by the enlarged fetal gonads for the production of estrogens and maternal C-21 precursors for the synthesis of progesterone and large quantities of 5α-reduced progestagens.[21] Near term, additional pregnenolone is secreted by the fetal adrenal glands[24,25] so that the mare exhibits the unusual phenomenon of foaling while maternal serum progestagen concentrations are increasing and estrogen concentrations are decreasing. Thus, the mare is unusual among domestic species in that progesterone is only prominent during the early stages of gestation while plasma concentrations become negligible from mid-gestation to just prior to parturition[26] when plasma concentrations begin to rise markedly.[21] Consequently, a few studies have investigated the merits of monitoring progestagens as markers of pregnancy well-being in the mare.

Earlier studies have reported that progestagen concentrations are elevated in maternal plasma of mares as normal parturition approaches[27] and in mares experiencing placental abnormalities leading to fetal losses or premature deliveries in late gestation.[28,29] In contrast, those mares that abort their pregnancies during the first four months of gestation tend to have low plasma progesterone concentrations.[30] Unfortunately, the absolute concentration of progesterone needed to maintain pregnancy at this stage of gestation has not been defined but typically circulating plasma concentrations greater than 2.0 ng/ml are thought to be necessary to support early pregnancy. While progesterone may be useful as an endocrine marker of pregnancy well-being during the early stages of gestation, it may have less potential during the second half of gestation due to lack of availability of commercial assays for the progestin metabolites found in systemic blood. Although luteal production of progesterone may persist beyond Day 120 of gestation, by Day 70 of gestation there is a measurable production of progesterone by the placenta.[21] By Day 200 of gestation the placenta is the only source of progesterone which is rapidly metabolized to form 5α-pregnane metabolites. There are approximately nine progestagens other than progesterone that are quantitatively more important and may reach plasma concentrations ranging from 5 to 50 ng/ml.[25] Two of these, 20α-hydroxy-5α pregnan-20-one (20α5P) and 5α-pregnane,3β,20α-diol (αβ-diol) may reach circulating concentrations as high as 500 ng/ml [25] which are not detectable by most conventional progesterone assays. Moreover, there are quantitative differences in the total progestagen concentrations between different breeds of horses but individual progestagens isolated from maternal plasma show remarkable consistency between breeds.[28,31]

Measurement of circulating progestins has been advocated as a means of diagnosing placental dysfunction and pending pre-term delivery in the mare. In a 2005 study, Ousey and colleagues measured plasma concentrations of a cohort of pregestagens during late gestation in mares with normal and compromised pregnancies.[23] Total progestagen plasma concentration was measured using a commercially available ELISA assay system (Immulite Progesterone, Euro/DPC Ltd., Llaberis, Wales, UK) while nine specific metabolites were quantified using gas chromatography-mass spectrometry (GC-MS). The outcome of these studies was that mares with placentitis had increased concentrations of pregnenolone (P5), a primary precursor to progesterone, and/or increased progesterone and several metabolites including 5α-pregnane-30,20 dione, 20α5P and αβ-diol.[23] The author speculated that this

increase was due to increased fetal production of P5 and/or P4 and increased metabolism in the utero-placental tissues in response to chronic stress. On the other hand, mares experiencing placental pathologies other than from infection had a mixed response and no clear link was demonstrated between maternal plasma concentrations of P4, or any of the other progestagens and the maintenance of pregnancy. Others have suggested that in addition to measurement of progestin concentrations, transrectal ultrasonography may also be used in the diagnosis of impending pregnancy loss. A study was undertaken to evaluate the accuracy in identifying mares with feto-placental compromise by experimentally induced placentitis using transrectal ultrasonography of caudal uterus in conjunction with the assessment of plasma progestin profiles.[32] The outcome of such a dual diagnostic approach revealed that 20 of 22 mares were correctly identified with respect to their pregnancy outcome. The merits of ultrasonography as a diagnostic tool will be a subject of discussion later in this review.

Estrogens

Estrogens are not essential for the maintenance of pregnancy in the mare during late gestation but pregnancy is prolonged if there is disruption of normal fetal function leading to abnormal estrogen production which may result in poor uterine contractility and blood flow.[33] The mare differs from other domestic species in that the feto-placental unit produces copious amounts of estrogens during the second half of pregnancy including estrone, estradiol 17β and the equine-unique ring B unsaturated estrogens, equilin and equilenin.[25,29] The primary source for the estrogen precursors (i.e., dehydroepiandrosterone) in the horse is the fetal gonads[33,34] and the aromatization of these androgen precursors occurs in the placenta.[25] While the role of estrogens in pregnancy maintenance and parturition in the mare is not well understood, a few investigations have indicated that estrogen may promote synthesis of prostaglandins, an increase in oxytocin receptors and myometrial gap junctions thereby facilitating a switch in the amplitude and frequency of uterine contractions as parturition approaches.[10] Other studies have demonstrated that uterine activity is elevated during the final week pre-partum and this activity is more pronounced during the nocturnal hours correlating with nocturnal increase in plasma estradiol 17□concentrations.[35-37] Whether estrogens could be employed as useful diagnostic indicators of pregnancy well-being in the mare has not been adequately explored. However, some practitioners from clinical experience have reported that total serum estrogen concentrations <1000 ng/ml from approximately Day 150 to Day 300 of gestation is indicative of fetal stress while concentrations <500 ng/ml is generally indicative of impending abortion and the pregnancy is probably unresponsive to treatment.[38] On the other hand, studies in late-term mares stressed by medical or surgical problems or induced abortions concluded that maternal and serum concentrations of estrogens (i.e., estrone sulfate) were not a sensitive indicator of fetal compromise or death and were observed only to decline after severe fetal stress or abortion was imminent or had a occurred.[29,39]

Relaxin

The placenta is the primary source of the small polypeptide hormone relaxin,[40] more specifically the placental trophoblast cells.[41-43] Relaxin is an important hormone of pregnancy, not only for facilitating uterine and mammary gland development, but also in the maturation of the cervix to facilitate normal delivery.[16] Relaxin has several important functions during pregnancy and at time of parturition. Relaxin is essential for the live delivery of pups in the rat[44,45] and pigs in the sow.[46,47] Furthermore, relaxin treatment has been reported to improve calving in beef[48,49] and dairy heifers by advancing the dilatation of the cervix and increasing pelvic area, thereby decreasing the incidence of dystocia.[50] Relaxin can be measured in systemic blood of a number of mammalian species in high concentrations[51-56] and thus has the potential to be a valuable clinical tool for both diagnosing placental and fetal well-being during late gestation. There is evidence that the aborting dog exhibits depressed serum relaxin prior to pregnancy loss.[52,57] In addition, women with symptoms of impending miscarriage in early pregnancy had lower concentrations of relaxin compared to women with normal pregnancies.[58,59] A similar pattern was observed in dogs exhibiting early embryonic loss.[60] This led to the suggestion that relaxin might be a useful epidemiological tool in predicting pregnancy outcome in women.[59] Little is known concerning the role of relaxin in equids during pregnancy and parturition. There is some evidence that relaxin secretory pattern is compromised during at risk pregnancies and complicated deliveries. Stewart and co-workers reported that plasma relaxin concentrations were low in Standardbred mares with abnormal termination of pregnancy and suggested that relaxin might be a useful indicator of placental function.[61] Others have observed improved relaxin profiles and pregnancy outcomes in pony mares with at-risk pregnancies following treatment for fescue toxicosis.[62]

A recent study reported findings from retrospective analyses of systemic relaxin in clinical cases (i.e., twin pregnancies, oligohydrallantois, hydrops, premature placental separation, placentitis) of mares presented with at-risk pregnancies and a controlled study where therapeutic strategies were evaluated in late gestation mares

experimentally infected to induce placentitis.[63] While relaxin profiles were found to be compromised in some of the clinical cases observed, the reliability of using circulating relaxin as a predictor for drug efficacy following the treatment of a threatened pregnancy due to uterine infections could not be determined satisfactorily due to high variability in relaxin values.[63] Furthermore, there is some variation among breeds with respect to relaxin profiles during pregnancy,[61] however, the functional significance of these differences in terms of actions of relaxin is unknown. In dogs, systemic relaxin near term varies considerably between individuals but lower than normal concentrations were associated with embryonic loss[60] and spontaneous abortion.[52,57] These data in dogs appear to support observations that individual mares with compromised placentas show variable systemic relaxin and this variability is sustained regardless of therapeutic intervention.[63] Accordingly, relaxin may be less reliable in predicting pregnancy outcome following therapeutic intervention but useful in assessing loss of placental function.

Assessment of feto-placental well-being by ultrasonography

Ultrasonography allows for the detailed and, to all intents and purposes, safe analyses of the function of the utero-placental unit and fetus during pregnancy. Imaging of the placenta and fetus began more than forty years ago with the initial introduction of the ultrasound with regular B-mode (2-dimensional grey-scale) which has evolved in the intervening years with the development and use of color Doppler, 3- and 4-dimensional imaging and the more recent introduction of contrast ultrasonography in human obstetrics and gynecology.[64] Ultrasonography is now routinely used in human clinics, not only for assessment of placental function and vascular development, but also for identifying disorders of pregnancy including fetal growth restriction, pre-eclampsia, hydrops, oligohydrallantois, and pathogenic infectious processes.[64] The same level of sophisticated technology is not as readily available to most veterinary practitioners due to cost of equipment, but ultrasonogrpahy is used in most practices for assessment of ovarian function, pregnancy determination, placental and fetal well-being in both domestic livestock and companion animal species.[65] Much of the breakthrough in the application of ultrasonography as it relates to pregnancy in the mare was undertaken by Ginther and colleagues in the early 1980s.[66-68] Moreover, anomalies of the utero-placental unit using abdominal and transrectal ultrasonography have been important factors in the evaluation and assessment of fetal well-being and pregnancy outcome in normal and at-risk pregnancies in cattle (see review),[69] horses and sheep.[70-73] Transrectal ultrasonography has been especially useful in identifying those mares with placental disorders (utero-placental thickening, placental separation, placentitis) or mares with pending abortion due to disease or non-pregnancy-related illness.[32,72,74] From a practitioner's perspective, transrectal ultrasongoraphy permits: 1) the measurement of the combined thickness of the uterus and placenta (CTUP) at the cervix, 2) evaluation of placental edema or premature separation, 3) evaluation of allantoic and amniotic fluids, and 4) observation of the amnion and the measurement of its thickness.[38] These parameters will be explored in more detail below.

Evaluation of changes in fetal fluid volume or echogenicity has proved to be of some diagnostic value. Ultrasonographic measurement of decreased fluid volume (oligohydrallantois) is an abnormal finding in the mare and is usually associated with poor pregnancy outcome.[71,75] Moreover, changes in the echogenicity of equine fetal fluids have been associated with various fetal diseases in foals including placentitis, mare reproductive loss syndrome (see reviews)[76,77] septicemia and peripartum asphyxia syndrome. However, echogencity in fetal fluids also increases as term approaches in normal pregnancies and thus may not be as useful a predictor of pregnancy well-being.[71] Amniotic fluid can be sampled to predict fetal lung maturity by evaluating the lecithin to sphyngomyelin ratio (L/S) or performing amniotic lamellar body counts. Although amniocentesis is regarded as a safe procedure to perform in pregnant women it is a much more difficult procedure to perform in late gestation domestic animals with a reported incidence of abortion of 8% in cattle[78] and as high as 25% in the mare.[79] Transvaginal ultrasound-guided aspiration is a technique currently used with reasonable success by some practitioners to reduce unilateral twin vesicles in the mare.[80] However, in our hands, both transabdominal and transcervical ultrasound-guided amniocentesis in the pony mare proved to be highly problematic leading to spontaneous abortion (D. Christiansen, personal communication). On a more positive note, ultrasonography can also be used to provide some predictable outcomes of pregnancy by measuring several biophysical parameters including fetal heart rate, fetal breathing movements, fetal body movements and measurements of specific organ parameters such as the stomach, heart, kidneys, fetal gonads, and fetal aortic and trachea diameter.[71,81,82]

In a comprehensive study to develop criteria for assessing feto-placental well-being in the mare, Bucca and colleagues employed both transrectal and transabdominal ultrasonography to monitor several biophysical parameters including evaluation of fetal heart rate, respiration, activity and assessment of vital organs, echogenicity and depth of fetal fluids and uteroplacental membrane thickness from mid-gestation to term.[82] These authors have provided a detailed profile of normal biophysical parameters of the fetus at monthly intervals from gestation month

six (150-180 days) through to gestation month 12 (330-360 days) that could be evaluated as useful prognostic parameters. An interesting observation was the steady decline in both the mean heart rate at rest and during activity. Other consistent markers were the steady increase in fetal aortic and orbital diameters. However, transrectal measurements of CTUP, which were obtained at the ventral aspect of the cervical pole, showed considerable variation but the mean ventral CTUP showed a consistent increase from month to month.[82] Renaudin and colleagues have reported a marked increase in CTUP in cases if ascending placentitis that were later confirmed by histopathology.[72] In a more recent study, Morris and coworkers measured endocrine markers (progestin profiles) in conjunction with transrectal ultrasonography, particularly the CTUP in mares with experimentally induced placentitis.[32] These investigators found a consistent relationship between placentitis and abnormal progestin profiles associated with an increase in CTUP above 1 cm. By performing the two tests, they reported that pregnancy outcomes were correctly predicted in twenty of the twenty two mares in the study.[32] Ultrasonography has now become standard practice for evaluation of reproductive function, pregnancy confirmation and feto-placental well-being in most equine practices and is often supported by endocrine analysis when evaluating pregnancy well-being.

Doppler ultrasonography

While Doppler ultrasonography has been around for some time as a means of assessing vascular blood flow to vital organs it has become in more recent times an important clinical tool for assessing placental performance in healthy and high-risk human pregnancies and useful in predicting later complications and outcome of pregnancies that otherwise appear uncomplicated.[83] This technology has been particularly useful in predicting pre-eclampsia and intrauterine growth restriction.[84,85] Doppler ultrasonography has the advantage as a clinical tool in that it is capable of assessing fetal (umbilical artery), maternal (uterine arteries) and placental circulations (intraplacental circulation). Doppler ultrasonography has been used to characterize uterine blood flow throughout gestation in a number of species including the cow,[86-88] dog[89] and cat.[90] A recent study characterized the functional hemodynamics of the utero-placental arterial vessels in the rabbit during pregnancy as well as in the umbilical cord, aorta and caudal vena cava of fetuses.[91] In this study, the authors observed throughout gestation a significant ($P < 0.05$) increase in the systolic peak velocity and end diastolic velocity in maternal and fetal vessels, whereas pulsatility index and resistance index decreased ($P < 0.05$), except in utero-placental vessels. This led the authors to suggest that the pregnant rabbit could be used as an experimental animal model to assess by Doppler ultrasonography, functional hemodynamic changes in placental and fetal vessels under both normal and pathophyisological conditions.[91]

In the equine, there has been limited use of this technology for diagnostic purposes of high-risk pregnancies since the initial report on the detection of fetal circulation in the mare and cow using Doppler ultrasonography.[92] However, its application and potential usefulness in the mare has been evaluated.[93] In contrast, much research has been undertaken in this field with regard to ovarian function, implantation and early fetal development.[94-96] A limiting factor in the past was the size of the equipment and the cost, but recent technological advances have led to the production of battery powered, hand-held color Doppler machines. However, the expense of these devices may still undermine the economical use of Doppler technology for most large animal practitioners.[88] To the best of our knowledge, there is no comprehensive report on the use of Doppler ultrasonography as a means of assessing fetal, placental and uterine circulation during late gestation in the mare, and whether such an approach would yield useful predictive information for determining pregnancy outcome is unknown. Thus, color Doppler ultrasonography is an area that may need further investigation as to its suitability as a diagnostic approach for assessing and identifying high-risk pregnancies during late gestation in the mare.

Qualitative and quantitative evaluation by ultrasound of fetal fluid, utero-placental unit and combined thickness of the uteroplacental unit and biophysical parameters such as fetal heart and respiration rates can be useful prognostic markers of feto-placental well-being. The most common clinical conditions requiring the need for assessment of feto-placental health in the mare are history of problematic pregnancies, placentitis, trauma, systemic illness or major surgery. However, it has been suggested that such measurements as a routine means of fetal monitoring is of limited benefit in the equine due to the inability to accelerate precocious fetal maturation and safely induce parturition.[82] In addition to the use of ultrasonography as a means of monitoring pregnancy well-being, major advances have also been made in utilizing other non-invasive approaches to identify novel biomarkers associated with compromised pregnancies.

Proteomic analysis of amniotic and cervico-vaginal secretions

Recent advances in proteomic technology have sparked interest in identifying protein biomarkers of preterm pregnancies in humans. The premise behind these studies is that the proteomic profile of unhealthy tissues is drastically different than that of normal tissues. From these studies, valuable knowledge and research techniques can be gained and possibly applied to preterm pregnancies in the equine. To this end, proteomic technology has the potential to allow for the discovery of preterm pregnancy biomarkers that would permit early detection of "at risk" pregnancies so that adequate medical intervention can be provided to the pregnant mare and fetus.

Sampling

In the search for preterm pregnancy biomarkers, one of the primary considerations is to utilize a safe and relatively non-invasive sampling technique that is capable of revealing differences between normal and compromised pregnancies without inflicting harm upon the pregnancy. Much of the literature surrounding the detection of preterm pregnancy biomarkers in humans utilizes either amniotic fluid or cervico-vaginal fluid (CVF) for proteomic analysis.[1,2,97-99] However, amniocentesis is a fairly invasive procedure and can increase the risk of miscarriage,[100,101] so if the goal is to utilize a minimally invasive sampling technique, then CVF would be the most ideal candidate to use in the mare. Cervico-vaginal fluid can be obtained non-invasively simply by swabbing the inside of the vaginal wall.[101] The swab is then placed into a polystyrene tube containing a buffer solution, and the contents of the tube are centrifuged, allowing the supernatant to be collected for storage at -80 °C or immediate analysis.[102] Accordingly, this approach would provide a non-invasive means for acquiring a diagnostic sample, avoiding the risks associated with performing amniocentesis while still providing a sample for proteomic analysis.

Proteomic analysis–a basic description

Once an adequate sample is collected, it must be subjected to proteomic analysis which allows for the isolation, detection and quantification of small amounts of proteins within a complex mixture of other proteins,[103] thus allowing protein expression patterns to be discovered when comparing normal and compromised pregnancies. The diagnostic sample is analyzed by one of the many proteomic techniques that exist. Generally, this is a two step process, whereby the samples are first subjected to a separation process, such as liquid chromatography or two-dimensional gel electrophoresis (2DGE), followed by analysis by one of the many mass spectrometry techniques (MS), such as matrix-assisted laser desorption/ionization time of flight (MALDI-TOF), electrospray ionization (ESI), or surface-enhanced laser desorption/ionization (SELDI) MS[104,105] which identify the chemical properties that are unique to every protein. The information from the MS analysis is compared to data stored in enormous bioinformatic databases, and the proteins from the sample are identified.[103] This is obviously an over-simplified description of the elaborate steps in the process, yet it provides a concise depiction of this technique. Ultimately, the goal is to characterize disorders, such as preterm birth, based upon the type and amount of protein present in a given sample in the hope of creating a panel of biomarkers that would be indicative of mares at risk of delivering early. The disadvantage is that veterinary clinics would have to submit samples to an appropriate diagnostic laboratory for processing and currently it is cost-prohibitive for use in general practice.

Current progress in human medicine

Often, human medical knowledge stems from work performed in animals; however, in the case of identifying proteomic biomarkers for pregnancy complications in the equine, such as preterm birth and intra-amniotic infection (IAI), much can be learned from the recent work involving clinical research in humans. One of the more promising studies was conducted by Buhimschi and colleagues in which amniotic fluid samples from 104 patients were evaluated.[105] Utilizing SELDI MS, these authors identified a unique panel of four proteins, comprised of neutrophil defensins-1 and -2 and calgranulins A and C, that was associated with women eventually diagnosed with IAI. Further work included assigning the patients a score value (ranging from 0 to 4), which gave the patients one point for each of the four identified proteins found in the patient's amniotic fluid.[105] For patients with a score > 2, IAI was diagnosed with 100% sensitivity and specificity; furthermore, patients with a score of 3 or 4 had a decreased time interval between amniocentesis and delivery.[105] Further clinical research utilizing the scoring system described by Buhimschi[105] revealed that patients with scores of 0, 1-2, and 3-4 gave birth to offspring with gestational ages of 32.1, 30.6, and 27.5 weeks, respectively.[106] Additionally, scores of 3-4 were associated with amniotic inflammation, the presence of gram staining bacteria in the amniotic fluid, and elevated white blood cell and interleukin-6 concentrations, all of which are indicative of IAI.[106] In a similar study,[97] proteomic analysis of amniotic fluid revealed that calgranulin B and a proteolytic fragment of insulin like growth factor binding protein-1 (IGFBP-1) were found to be present in the amniotic fluid of patients with IAI but absent in non-infected patients,[97] revealing two additional potential protein biomarkers of IAI in humans. There are also data to support the use of

fetal fibronectin in evaluating preterm patients, especially those with symptomatic contractions and minimal cervical dilation.[107] Fetal fibronectin found in cervico-vaginal secretions was found to be a sensitive marker for risk of preterm delivery in women although the predictive value was not found to be high; rather it was found to be more reliable when values were negative that risk of preterm delivery is low.[107]

Collectively, these findings validate the role that proteomic analysis of amniotic fluid may have in identifying biomarkers of compromised pregnancies; however, proteomic analysis of CVF has also been successful in identifying potential biomarkers,[1,2,98] making CVF a more appealing sampling method for equine studies due to its non-invasive nature. Recently, Gravett and colleagues established IAI in four rhesus monkeys by intra-amniotic inoculation of *U. parvum*.[98] Proteomic analysis of CVF samples revealed that among the many proteins found to be present in the CVF proteome, calgranulin A and B, annexin II, and a proteolytic fragment of IGFBP-1 were expressed more in the CVF samples taken after inoculation,[98] providing results similar to those observed in amniotic fluid of IAI patients.[97,105] Moreover in an elaborate study, Pereira and coworkers characterized not only the CVF proteome of patients with term and preterm births but also the proteome of CVF in patients experiencing only symptoms of preterm labor but delivered at term.[2] Using multiple proteomic approaches, different protein expression patterns were noted between women with normal pregnancies and those who experienced symptoms of preterm labor.[2] Interestingly, comparisons of the CVF proteome revealed that calgranulins A, B and C, annexin V, and proteolytic fragments of IGFBP-1 were up-regulated in women with preterm birth when compared to women with only preterm labor symptoms, and further comparisons demonstrated that these proteins were also up-regulated in cases of preterm birth when compared to term pregnancies.[2] The fact that there are different protein signatures between the CVF of women with symptoms of preterm labor and preterm birth[2] suggests that a diagnostic test could be developed to distinguish between those patients that experience symptoms of preterm labor but carry the pregnancy to term and those patients that present with preterm labor and experience preterm birth. This approach has great potential as a diagnostic tool for identifying "at-risk" pregnancies in the equine and we are currently performing preliminary studies to determine this possibility.

Physiological role of differentially expressed proteins

Identifying proteins that are up-regulated in compromised pregnancies is an important step towards fully describing the pathophysiology associated with preterm birth; however, understanding the physiological function of these proteins is also crucial. Among the many proteins that have been identified as potential biomarkers of preterm pregnancies, many of which are not mentioned in this paper, there appear to be eight which are consistently associated with the occurrence of preterm birth and IAI in humans: annexins II and V, proteolytic fragments of IGFBP-1, neutrophil defensins 1and 2, and calgranulins A, B, and C[2,97,98105,106] It is plausible that these eight proteins may one day comprise a panel of biomarkers to aid in the diagnosis of preterm pregnancies; therefore, discussion of their function in relation to preterm pregnancies and IAI cannot be disregarded.

Annexins are a group of proteins that have a wide variety of physiological functions within cells, including inflammatory and defense responses.[98] By binding to calcium and phospholipids,[108] annexins regulate prostaglandin formation, a process that involves the mobilization of arachidonic acid from cell membrane phospholipids through the action of phospholipase A2, which requires calcium for proper function.[109,110] Thus, through regulation of annexin protein expression, prostaglandin production can also be controlled throughout pregnancy and at parturition. In cases of IAI, annexins are up-regulated[98] possibly in response to invading pathogens; however, for preterm birth without infection, an explanation for an increase in annexins in CVF[2] cannot be offered, yet it should be noted that if these proteins are irregularly expressed at inappropriate times during gestation, abnormalities in prostaglandin production and preterm birth cannot be ruled out as consequences.

Throughout gestation, the expression of insulin-like growth factor (IGF) is necessary for many events including implantation and fetal growth.[111,112] In addition to IGF, IGFBP-1, localized in the decidua,[113] is also vital for a successful pregnancy through its regulation of IGF activity.[112] The presence of IGFBP-1 in amniotic fluid would not be unexpected; however, cases of IAI in which a proteolytic fragement of IGFBP-1 was found to be over-expressed in amniotic fluid[97] and CVF[2] should cause concern. The presence of an IGFBP-1 proteolytic fragment in the amniotic fluid and CVF has been suggested to be indicative of a response to infection that involves a protease-related mechanism.[98] Furthermore, the presence of an IGFBP-1 proteolytic fragment in the CVF is of great concern because it signifies a failure of tissue junctions located at the choriodecidual interface, allowing chorionic-decidual products to leak into the vaginal cavity.[113] Therefore, the presence of proteolytic fragments of IGFBP-1 in the amniotic fluid[97] and CVF[2] of patients with IAI can be explained as a response to invading pathogens that causes the cleavage of IGFBP-1, and additionally, the presence of an IGFBP-1 proteolytic fragment in the CVF would indicate a failure of membrane junctions, possibly preceding preterm birth.

Neutrophil defensins 1 and 2 are small cationic peptides that are stored in the primary granules (azurophil) of neutrophils[114] and are known to provide immune protection from bacterial, fungal and viral infections.[115] Information surrounding neutrophil defensins suggests that these antimicrobial proteins exert their antimicrobial activity through a non-oxidative process,[114] whereby they disrupt the structural integrity of the microbial cell membrane, ultimately causing cell death.[116,117] Additionally, neutrophil defensins provide a link between innate and adaptive immunity through their recruitment of adaptive immune cells such as monocytes and T-cells, leading to a prolonged immune response.[114,117] Thus, it is not surprising to find these two proteins (neutrophil defensins 1 and 2) up-regulated in the amniotic fluid of patients with IAI.[105,106] The presence of these proteins suggest an immune response by the patient's body that will provide both an immediate action, through the antimicrobial properties of defensins, and a long term immune response through the recruitment of adaptive immune cells such as monocytes and T-cells.

In addition to defensin proteins, calgranulins A, B, and C are also expressed in neutrophils and are classified as members of the S100 protein family.[114] Calgranulin C is considered to be a minor calgranulin in neutrophils but still provides bactericidal properties against gram-negative bacteria.[114] Together, calgranulins A and B form a zinc and calcium binding heterodimer protein referred to as calprotectin,[118] which exerts a biostatic effect on a wide variety of microorganisms by competing against microorganisms for calcium and zinc, which are vital for cellular activity.[114,119] Furthermore, in addition to its antimicrobial properties, calgranulin B is also implicated in the normal process of parturition given that its expression level increases in the cervix and myometrium at the onset of term labor.[118] Additionally, calgranulin A and B play a role in prostaglandin production by promoting arachidonic acid transport in the cervix and myometrium during normal parturition.[118] Understanding the physiological function of calgranulins A, B, and C provides insight into why they are up-regulated in cases of IAI and preterm birth.[2,97,98,105.106] For cases of IAI, the up-regulation of the calgranulins provides an immediate immune response to invading pathogens by competing for minerals that are vital for the pathogen's growth and survival. Additionally, in cases of preterm birth, premature up-regulation of calgranulins A and B possibly leads to an untimely increase in prostaglandins, ultimately inducing labor before term.

From the studies described, it is apparent that protein biomarkers of preterm birth and IAI are present in the amniotic fluid and cervico-vaginal fluid of humans.[2,97,98,105,106] This affords many opportunities for equine researchers and clinicians to apply proteomic technology to the study of equine preterm birth in the hope of identifying similar protein biomarkers for preterm birth. Ultimately, the goal would be to utilize the information from such studies for the development of simple and rapid screening tests that would afford early detection of mares at risk of delivering before term so that adequate medical intervention can be provided to the mare and developing fetus.

Biophotonic imaging technology and pathogenesis in reproduction

Biophotonics involves the union of photonics and biology, and deals with the interactions between light, biological matter and its application to biomedical science. Nature has harnessed the photon (light) in many ways as a basic principle of life; whether through photosynthetic pathways in plants, as methods of communication among insects (e.g., firefly) or a multitude of other examples from across the natural world. The use of photonics in experimental models of human conditions has become a key diagnostic and research tool for understanding physiological systems in normal and diseased states that were not previously attainable with other detection systems. The last 10 to15 years has seen unprecedented adaptations of this technology for investigating a variety of physiologically relevant systems in situ, including single cell,[120-122] whole plants,[123] Drosphila[124] and rodents.[125-127] Real time imaging technology using luciferase reporter genes to monitor physiologcal events *in vivo* has become an important and meaningful approach to studying, in real time and over time, biological responses including efficacy of drugs for cancer therapy, pathogenesis of bacterial pathogens or the regulation and expression of specific genes during specific physiological events.[128]

Recently, we have employed a transgenic mouse model[129] in which a promoter region of the vascular endothelial growth factor receptor 2 (VEGFR2) gene, is cloned up stream of the luciferase gene and when activated, in the presence of exogenous substrate (i.e., luciferin), can be non-invasively monitored using a highly sensitive imaging system to study regulation of vascular development under different physiologcial conditions.[127,130] The VEGFR2 gene is transcriptionally regulated during angiogenesis under the influence of VEGF, an important angiogenic peptide.[131] Currently, we are employing this mouse model to investigate the effects of intra-uterine growth restriction as a consequence of dietary deficiencies in pregnant females by monitoring the expression pattern of the VEGFR2-lux gene in fetal-placental tissues (Greene and Ryan, 2010, unpublished observations). Recent applications of biophotonic paradigms in large animal models including swine[132] and sheep,[133,134] and the development of methods to facilitate deep tissue photon capture (e.g., optical clearing techniques)[135] across tissues

(skin and intestine) suggests *in vivo* as opposed to *ex vivo* or post-mortem imaging may be feasible in the near future using minimally invasive detection procedures. Moreover, we have demonstrated the benefits of using genetically modified bacteria with the lux operon and bioluminescence imaging technology for evaluating pathogen progression in the gastro-intestinal tract of swine,[132] in *ex vivo* studies of fetal lambs delivered to infected ewes[133] and the *ex vivo* bovine reproductive tract.[136] Through further development of the paradigm outlined in the above reported studies, applications might be extended to provide a more resolved model for understanding the progression of events (e.g., bacterial-induced endocrine changes, bacterial invasiveness of the fetal environment) that leads to preterm delivery and pregnancy failure in mares, and may have applications for evaluation of therapeutic interventions with the goal of reducing antenatal mortality.[137] In addition, the postpartum clearance of bacteria following abortion and therapeutic intervention is another potentially valuable application for this technology.[137]

Conclusion

This review explored the merits and disadvantages of present day diagnostics used as indicators of pregnancy well-being and introduced the potential application of new and/or emerging techniques currently employed in human medicine (i.e., proteomic marker analayses of amniotic and cerivco-vaginal fluids). In addition, the potential merits of utilizing bioluminescence imaging technology to better understand the pathogenic process that lead to compromised pregnancies warrants continued investigation. The application of this novel imaging technology with *lux*-modified organisms may facilitate not only the development of more targeted therapeutic interventions, but also better diagnostic approaches to assess fetal and placental well-being. In addition, the ability to utilize cervico-vaginal swabs for evaluating pregnancy well-being in the mare has exciting possibilities in moving the diagnostic process forward. Ultimately, identifying "at risk" pregnancies in a timely manner may require a combinatorial approach using a number of emerging and current diagnostic tools as reliable predictors of pregnancy outcome.

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References

1. Shah SJ, Yu KH, Sangar V, et al: Identification and quantification of preterm birth biomarkers in human cervicovaginal fluid by liquid chromatography/tandem mass spectrometry. J Proteome Res 2009;8:2407-2417.
2. Pereira L, Reddy AP, Jacob T, et al: Identification of novel protein biomarkers of preterm birth in human cervical–vaginal fluid. J Proteome Res 2007;6:1269-1276.
3. Butt RH, Lee MWY, Pirshahid SA, et al: An initial proteomic analysis of human preterm labor: placental membranes. J Proteome Res 2006;5:3161-3172.
4. Mazaki-Tovi S, Romero R,Vaisbuch E, et al: Adiponectin in amniotic fluid in normal pregnancy, spontaneous labor at term, and preterm labor: a novel association with intra-amniotic infection/inflammation. J Matern Fetal Neonatal Med 2010;23:120-130.
5. Romero R, Mazaki-Tovi S, Vaisbuch E, et al: Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med. 2010 May 26. [Epub ahead of print]PMID: 20504069.
6. Vaala WE, Sertich PL: Management strategies for mares at risk for periparturient complications. Vet Clin North Am Equine Pract 1994;10:237-265.
7. Giles RC, Donahue JM, Hong CB, et al: Causes of abortion, stillbirth and perinatal death in horses: 3,527 cases (1986-1991). J Am Vet Med Assoc 1993;203:1170-1175.
8. Hong CB, Donahue JM, Giles RC, et al: Etiology and pathology of equine placentitis. J Vet Diag Invest 1993;5:56-63.
9. Whitwell KE: Infective placentitis in the mare. In Powell DG, editor. Equine infectious diseases V: Proc 5th Int Conf. Lexington:University of Kentucky Press; 1988. p. 172-180.
10. LeBlanc MM: Ascending placentitis in the mare: an update. Reprod Domest Anim 2010; In press.
11. Redmond LM, Cross DL, Strickland JR, et al: Efficacy of domperidone and sulpiride as treatments for fescue toxicosis in horses. Am J Vet Res 1994;55:722-729.
12. Cross DL, Redmond, LM, Strickland JR: Equine fescue toxicosis: signs and solutions. J Anim Sci 1995;73:899-908.
13. Bennet-Wimbush K, Loch W: A preliminary study on the efficacy of fluphenazine as a treatment for fescue toxicosis in gravid pony mares. J Equine Vet Sci 1998;18:169-173.
14. LeBlanc MM: Identification and treatment of the compromised equine fetus: a clinical perspective. Equine Vet. J 1997;24(Suppl):74-82.
15. Bailey CS, Macpherson ML, Pozor MA: Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in experimentally induced equine placentitis. 2010; doi:10.1016/j.theriogenology.2010.02.023
16. Sherwood OD: Relaxin. In Knobil E, Neill JD, editors. The physiology of reproduction. 2nd ed. New York: Raven Press; 1994. p. 861-1009.
17. Terqui M, Palmer E: Oestrogen pattern during early pregnancy in the mare. J Reprod Fertil 1979; 27:441-446.

18. Stabenfeldt GH, Daels PF, Munro CJ, et al: An oestrogen conjugate enzyme immunoassay for monitoring pregnancy in the mare. J. Reprod. Fertil 1991;44(Suppl):37-44.
19. Allen WR. Luteal deficiency and embryo mortality in the mare. Reprod Domest Anim 2001;36:121-131.
20. Silver M. Placental progestagens in the sheep and horse and the changes leading to parturition. Exp Clin Endocrinol 1994;102:203-211.
21. Allen WR. Fetomaternal interactions and influences during equine pregnancy. Reproduction 2001;121:513-527.
22. Ousey JC, Forhead AJ, Rossdale PD, et al: The ontogeny of uteroplacental progestagen production in pregnant mares during the second half of gestation. Reproduction 2003;69:540-548.
23. Ousey JC, Houghton ED, Grainger L, et al: Progestagen profiles during the last trimester of gestation in Thoroughbred mares with normal or compromised pregnancies. Theriogenology 2005;63:1844-1856.
24. Fowden AL, Forhead AJ, Ousey JC: The endocrinology of equine parturition. Exp Clin Endocrinol Diabetes 2008;116:393-403.
25. Ousey JC: Peripartal endocrinology in the mare and foetus. Reprod Domest Anim 2004;39:222-231.
26. Ginther OJ: Reproductive biology of the mare. 2nd ed. Cross Plains (WI): Equiservices; 1992. p. 419-456.
27. Hamon M, Clarke SW, Houghton E, et al: Production of 5α-dihydroprogesterone during late pregnancy in the mare. J Reprod Fertil 1991;44(Suppl):529-535.
28. Rossdale PD, Ousey JC, Cottrill CM, et al: Effects of placental pathology on maternal plasma progestagen and mammary secretion calcium concentrations and on neonatal adrenocortical function in the horse. J Reprod Fertil 1991;44(Suppl):579-590.
29. Santschi EM, LeBlanc MM, Weston PG: Progestagen, oestrone sulphate and cortisol concentrations in pregnant mares during medical and surgical disease. J Reprod Fertil 1991;44(Suppl):627-634.
30. Daels PF, Besognet B, Hansen B, et al: Effect of progesterone on prostaglandin F2α secretion and outcome of pregnancy during cloprostenol-induced abortion in mares. Am J Vet Res 1996;57:1331-1337.
31. Holtan DW, Houghton E, Silver M, et al: Plasma progestagens in the mare, fetus and newborn foal. J Reprod Fertil 1991;44(Suppl):517-528.
32. Morris S, Kelleman AA, Stawicki RJ, et al: Transrectal ultrasonography and plasma progestin profiles identifies feto-placental compromise in mares with experimentally induced placentitis. Theriogenology 2007;67:681-691.
33. Pashen RL, Allen WR: The role of the fetal gonads and placenta in steroid production, maintenance of pregnancy and parturition in the mare J Reprod Fertil 1979;27(Suppl):499–509.
34. Raeside JI, Liptrap RM: Patterns of urinary oestrogen excretion in individual pregnant mares. J Reprod Fertil 1975;23(Suppl):469-475.
35. Haluska GJ, Curie WB: Variations in plasma concentrations of oestradiol 17β and their relationship to those of progesterone 13,14-dihydro15-ketoprostaglandin F2α and oxytocin across pregnancy and at parturition in pony mares. J Reprod Fertil 1988;84:635-646.
36. O'Donnell LJ, Sheerin BR, Hendry JM, et al: 24-hour secretion patterns of plasma oestradiol 17beta in pony mares in late gestation. Reprod Domest Anim 2003;38:233-235.
37. Riddle WT: Preparation of the mare for normal parturition. Proc Ann Conv Am Assoc Equine Pract 2003. p. 1-5.
38. Madej A, Kindahl H, Mydahl C, et al. Hormonal changes associated with induced late abortions in the mare. J Reprod Fertil 1987;35(Suppl):479.
39. McGlothlin JM, Lester G, Hansen PJ, et al: Alteration in uterine contractility in mares with experimentally induced placentitis. Reproduction 2004;127:57-66.
40. Stewart DR, Stabenfeldt GH, Hughes JP, et al: Determination of the source of equine relaxin. Biol Reprod 1982;27:17-24.
41. Klonisch T, Ryan PL, Yamashiro S, et al: Partial complementary deoxyribonucleic acid cloning of equine relaxin messenger ribonucleic acid, and its localization within the equine placenta. Biol Reprod 1995;52:1307-1315.
42. Klonisch T, S Mathias, Cambridge G, et al: Placental localization of relaxin in the pregnant mare. Placenta 1997;18:121-128.
43. Klonisch T, Hombach-Klonisch S: Review: relaxin expression at the feto-maternal interface. Reprod Domest Anim 2000;35:149-152.
44. Downing SJ, Sherwood OD: The physiological role of relaxin in the pregnant rat. I. The influence of relaxin on parturition. Endocrinology 1985;116:1200-1205.
45. Lao-Guico-Lamm ML, Sherwood OD: Monoclonal antibodies specific for rat relaxin. II. Passive Immunization throughout the second half of pregnancy disrupts birth in intact rats. Endocrinology 1998;123:2472-2478.
46. Nara BS, Welk FA, Rutherford JE, et al: Effect of relaxin on parturition and frequency of live births in pigs. J Reprod Fertil 1982;66:359-365
47. Watts AD, Flint APF, Foxcroft GR, et al: Plasma steroid, relaxin and dihydro-keto-prostaglandin F-2 alpha changes in the mini-pig in relation to myometrial electrical and mechanical activity in the pre-partum period. J Reprod Fertil 1998;83:553-564.
48. Musah AI, Schawbe C, Willham RL, et al: Relaxin induction of parturition in beef heifers. Endocrinology 1986;118:1476-1482.
49. Musah AI, Schawbe C, Willham RL, et al: Dystocia, pelvic and cervical dilatation in beef heifers after induction of parturition with relaxin combined with clorprostenol or dexamethasone. Anim Reprod Sci 1988;16:237-365.
50. Bagna B, Schwabe C, Anderson L: Effect of relaxin on facilitation of parturition in dairy heifers. J Reprod Fertil 1991;91:605-615.
51. Niemuller CA, GrayC, Cummings E, et al: Plasma concentration of immunoreactive relaxin activity and progesterone in the pregnant Asian elephant (*Elephas maximus*). Anim Reprod Sci 1998;53:119-131.
52. Steinetz BG, Bullesbach EE, Goldsmith LT, et al: 1996. Use of a synthetic canine relaxin to develop a rapid homologous radioimmunoassay. Biol Reprod 1996;54:1252-1260.
53. Steinetz BG, Brown JL, Roth TL, et al: Relaxin concentrations in serum and urine of endangered species. Ann NY Acad Sci 2004;1041:367-378.
54. Steinetz B, Lasano S, de Haas F, et al: Relaxin concentrations in serum and urine of endangered and crazy mixed-up species: new methods, uses and findings. Ann NY Acad Sci 2009;1160:179-185.
55. Stewart DR: Development of a homologous equine relaxin radioimmunoassay. Endocrinology 1986;119:1100-1104.
56. Bravo PW, Stewart DR, Lasley BL, et al: Hormonal indicators of pregnancy in llamas and alpacas. J Am Vet Med Assoc 1996;208:2027-2030.
57. Schafer-Somi S, Aksoy OA, Beceriklisoy HB, et al: Repeated induction of abortion in bitches and the effect on plasma concentrations of relaxin, progesterone and estradiol 17β. Theriogenology 2007;68:889-895.

58. Witt BR, Wolf GC, Wainwright C, et al: Relaxin, CA-125, progesterone, estradiol, Schwangerschaft protein, and human chorionic gonadotropin as predictors of outcome in threatened and non-threatened pregnancy. Fertil Steril 1990; 53:1029-1036.
59. Stewart DR, Overstreet JW, Celniker AC, et al: The relationship between hCG and relaxin secretion in normal pregnancies vs peri-implantation spontaneous abortions. Clin Endocrinol (Oxf) 1993;38:379-385.
60. Gunzel-Apel AR, Zabel S, Bunk CF, et al: Concentrations of progesterone, porlactin and relaxin in the luteal phase and pregnancy in normal and short-cycling German Shepherd dog. Theriogenology 2006;66:1431-1435
61. Stewart, DR, Addiego LA, Pascoe DR, et al: Breed difference in circulating equine relaxin. Biol Reprod 1992;46:648-652.
62. Ryan PL, Bennet-Wimbush KW, Vaala WE, et al: Systemic relaxin in pregnant pony mares grazing on endophyte-infected fescue: effects of fluphenazine treatment. Theriogenology 2001;56:471-483.
63. Ryan PL, Christiansen DL, Hopper RM, et al: Evaluation of systemic relaxin blood profiles in horses as a means of assessing placental function in high-risk pregnancies and responsiveness to therapeutic strategies. Ann NY Acad Sci 2009;1160: 169–178.
64. Abramowicz JS, Sheiner E: In utero imaging of the placenta: importance for diseases of pregnancy. Placenta 2007;28 Suppl A:S14-22
65. Ginther OJ: Ultrasonic imaging and animal reproduction: fundamentals. Book 1. Cross Plains (WI): Equiservices; 1995. p. 71-73.
66. Ginther OJ: Mobility of the early equine embryo. Theriogenology 1983;19:603-611.
67. Ginther OJ: Fixation and orientation of the early equine conceptus. Theriogenology 1983;19:613-623.
68. Ginther OJ. Ultrasonic imaging and animal reproduction: horses. Book 2. Cross Plains (WI): Equiservices; 1995. p. 23-118.
69. Sebastien MC, Buczinskis GF, Lefebvre RC, et al: Fetal well-being assessment in bovine near term gestations; current knowledge and future perspectives arising from comparative medicine. Can Vet J 2007;48:178-183.
70. Reef VB, Vaala WE, Leila TW, et al: Ultrasonographic evaluation of the fetus and intrauterine environment in healthy mares during late gestation. Vet Radiol Ultrasound 1995;36:533-541.
71. Reef VB, Vaala WE, Worth LT, et al. Ultrasonographic assessment of fetal well-being during late gestation: development of an equine biophysical profile. Equine Vet J 1996;28:200-208.
72. Renaudin CD, Troedsson MHT, Gillis CL, et al: Ultrasonographic evaluation of the equine placenta by transrectal and transabdominal approach in the normal pregnant mare. Theriogenology 1997;47:559-573.
73. Renaudin CD, Troedsson MHT, Schrenzel MD: Transrectal ultrasonographic diagnosis of ascending placentitis in the mare: a report of two cases. Equine Vet Educ 1999;11:69-74.
74. Troedsson MHT, Renaudin CD, Zent WW, et al: Transrectal ultrasonography of the placenta in normal mares and mares with pending abortion: a field study. Proc Annu Conv Am Assoc Equine Pract 1997. p. 256-258.
75. Reimer JM: Use of transcutaneous ultrasonography in complicated latter-middle to late gestation pregnancies in the mare: 122 cases. Proc Am Assoc Equine Pract 1997. p. 259-261.
76. Sebastian MM, Bernard WV, Riddle TW, et al: Review paper: mare reproductive loss syndrome. Vet Pathol. 2008;45:710-722.
77. McDowell KJ, Webb BA, Williams NM, et al: Invited review: the role of caterpillars in mare reproductive loss syndrome: a model for environmental causes of abortion. J Anim Sci 2010;88:1379-1387.
78. Callan RJ, Schanckel JA, Van Campen H, et al: Percutaneous collection of fetal fluids for detection of bovine viral diarrhea virus in cattle. J Am Vet Med Assoc 2002;220:1348-1352.
79. Schmidt AR, Williams MA, Carleton CL, et al: Evaluation of transabdominal ultrasound-guided amniocentesis in the late gestational mare. Equine Vet J 1991;23:261-265.
80. Govaere JL, Hoogewijs MK, de Schauwer C, et al: Transvaginal ultrasound-guided aspiration of unilateral twin gestation in the mare. Equine Vet J 2008;40:521-522.
81. Pantaleon LG, Bain FT, Zent W, et al: Equine fetal growth and development. Compend Contin Educ Pract Vet 2003;25:470-477.
82. Bucca S. Fogarty U, Collins A, et al: Assessment of feto-placental well-being in the mare from mid-gestation to term: transrectal and transabdominal ultrasonographic features. Theriogenology 2005;64:542-557.
83. Abramowicz JS, Sheiner E: Ultrasound of the placenta: a systemic approach. Part II: Functional assessment (Doppler). Placenta 2008;29:921-929.
84. Cnossen JS, Morris RK, Terriet G, et al: Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. CMAJ 2008;178:710-711.
85. Hutchinson ES, Brownhill P, Jones NW, et al: Utero-placental haemodynamics in the pathogenesis of pre-eclampsia. Placenta 2009;30:63:634-641.
86. Bollwein H, Baumgartner U, Stolla R: Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. Theriogenology 2002;57:2053-2061.
87. Panarace M, Garnil C, Marfil M, et al: Transrectal Doppler sonography for evaluation of uterine blood flow throughout pregnancy in 13 cows. Theriogenology 2006;66:2113-2119.
88. Herzog K, Bollwein H: Application of Doppler ultrasonography in cattle reproduction. Reprod Domest Anim 2007;42:51-58.
89. Di Salvo P, Bocci F, Zelli R, et al: Doppler evaluation of maternal and foetal vessels during normal gestation in the bitch. Res Vet Sci 2006;81:382-388.
90. Scotti L, Di Salvo P, Bocci F, et al: Doppler evaluation of maternal and foetal vessels during normal gestation in queen. Theriogenology 2008;69:1111-1119.
91. Polisca A, Scotti L, Orlandi R, et al: Doppler evaluation of maternal and fetal vessels during normal gestation in rabbits. Theriogenology 2010;73:358-366.
92. Mitchell D: Detection of foetal circulation in the mare and cow by Doppler ultrasound. Vet Rec 1973;93:356-358.
93. Ginther OJ, Matthew D: Doppler ultrasound in equine reproduction: principles, techniques, and potential. J Equine Vet Sci 2004;24:516-526.
94. Bollwein H, Stolla R, Rohr S, et al: Relationships between uterine blood flow, estrogens and nitric oxide in cycling mares. Theriogenology 2002;58:575-578.
95. Bollwein H, Mayer R, Stolla R: Transrectal Doppler sonography of uterine blood flow during early pregnancy in mares. Theriogenology 2003;60:597-605.
96. Silva LA, Gastal EL, Berg MA, et al: Changes in vascular perfusion of the endometrium in association with changes in location of the embryonic vesicle in mares. Biol Reprod 2005;72:755-761.
97. Gravett MG, Novy MJ, Rosenfeld RG, et al: Diagnosis of intra-amniotic infection by proteomic profiling and identification of novel biomarkers. JAMA 2004;292:462-469.

98. Gravett MG, Thomas A, Schneider KA, et al: Proteomic analysis of cervical–vaginal fluid: Identification of novel biomarkers for detection of intra-amniotic infection. J Proteome Res 2007;6:89-96.
99. Rüetschi U, Rosén Å, Karlsson G, et al: Proteomic analysis using protein chips to detect biomarkers in cervical and amniotic fluid in women with intra-amniotic inflammation. J Proteome Res 2005;4:2236-2242.
100. Mukhopadhyay R: Biomarkers for preterm birth. J Proteome Res 2005;4:1900-1900.
101. Cottingham K: Research Profile: The cervical–vaginal fluid proteome and possible biomarkers of preterm birth. J Proteome Res 2007;6:1241-1242.
102. Di Quinzio MKW, Georgiou HM, Holdsworth-Carson SJ, et al: Proteomic analysis of human cervico–vaginal fluid displays differential protein expression in association with labor onset at term. J. Proteome Res 2008;7:1916-1921.
103. Thadikkaran L, Crettaz D, Siegenthaler MA, et al: The role of proteomics in the assessment of premature rupture of fetal membranes. Clin Chim Acta 2005;360:27-36.
104. Aebersold R, Mann M: Mass spectrometry-based proteomics. Nature 2003;422:198-207.
105. Buhimschi IA, Christner R, Buhimschi CS: Proteomic biomarker analysis of amniotic fluid for identification of intra-amniotic inflammation. BJOG 2005;112:173-181.
106. Buhimschi C, Dulay A, Abdel-Razeq S, et al: Fetal inflammatory response in women with proteomic biomarkers characteristic of intra-amniotic inflammation and preterm birth. BJOG 2009;116:257-267.
107. Yeast JD, Lu G: Biochemical markers for the prediction of preterm delivery. Clin Perinatol 2007;34:573-586.
108. Gerke V, Moss SE: Annexins: from structure to function. Physiol Rev 2002;82:331-371.
109. Lappas M, Rice GE: Phospholipase A2 isozymes in pregnancy and parturition. Prostaglandins Leukot Essent Fatty Acids 2004;70: 87-100.
110. Myatt L, Hirth J, Everson WV: Changes in annexin (lipocortin) content in human amnion and chorion at parturition. J Cell Biochem 1992;50:363-373.
111. Kutsukake M, Ishihara R, Yoshie M, et al: Involvement of insulin-like growth factor-binding protein-related protein 1 in decidualization of human endometrial stromal cells. Mol Hum Reprod 2007;13:737-743.
112. Nayak NR, Giudice LC: Comparative biology of the IGF system in endometrium, decidua, and placenta, and clinical implications for foetal growth and implantation disorders. Placenta 2003;24:281-296.
113. Rahkonen L, Rutanen E-M, Nuutila M, et al: Elevated levels of decidual insulin-like growth factor binding protein-1 in cervical fluid in early and mid-pregnancy are associated with an increased risk of spontaneous preterm delivery. BJOG 2010;117:701-710.
114. Harder J, Glaser R, Schroder J-M: Review: Human antimicrobial proteins effectors of innate immunity. J Endotoxin Res 2007;13:317-338.
115. Lehrer RI: Primate defensins. Nat Rev Microbiol 2004;2:727-738.
116. Ganz T: Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol 2003;3:710-720.
117. Selsted ME, Ouellette AJ: Mammalian defensins in the antimicrobial immune response. Nat Immunol 2005;6:551-557.
118. Kostakis ID, Cholidou KG, Kallianidis K, et al: The role of calprotectin in obstetrics and gynecology. European J Obst Gyn Reprod Biol 2010; In Press, Corrected Proof. doi:10.1016/j.ejogrb.2010.03.006.
119. Sohnle PG, Hunter MJ, Hahn B, et al: Zinc-reversible antimicrobial activity of recombinant calprotectin (migration inhibitory factor-related proteins 8 and 14). J Infect Dis 2000;182:1272-1275.
120. Willard ST, Faught WJ, Frawley LS: Real-time monitoring of estrogen-regulated gene expression from single, living human breast cancer cells: a new paradigm for the study of molecular dynamics. Cancer Res 1997;57:4447-4450.
121. Willard ST, Amstutz MD, Abrahmam E, et al: Simultaneous activity measurements of different hormonal genes in the same, living cell: Identification of mammosomatotropes. Am J Physiol Endocrinol Metab 1999;277:E1150-E1153.
122. Willard ST, Frawley LS: Real-time analysis of dynamic regulatory processes within living endocrine cells: In: Roubos EW, Wenderlaar-Bona SE, Vaudry H, et al, editors. Recent developments in comparative endocrinology and neurobiology. Maastricht, The Netherlands: Shaker Publishing; 1999.
123. Anderson SL, Kay SA: Functional dissection of circadian clock- and phytochrome-regulated transcription of the Arabidopsis CAB2 gene. Proc Natl Acad Sci 1995;92:1500-1504.
124. , Plautz JD, R. Stanewsky R, et al: Novel features of drosophila period transcription revealed by real-time luciferase reporting. Neuron 1996;16:687-692.
125. Contag CH, Contag PR, Mullins JI, et al: Photonic detection of bacterial pathogens in living hosts. Mol Microbiol 1995;18:593-603.
126. Contag CH, Spillamn SD, Contag PR, et al: Visualizing gene expression in living mammals using a bioluminescent reporter. Photochem Pathobiol 1997;66:5523-5531.
127. Ryan PL, Youngblood RC, Harvill J, et al: Photonic monitoring in real time of vascular endothelial growth factor receptor 2 gene expression under relaxin-induced conditions in a novel murine wound model. Ann NY Acad Sci 2005;1041:398-414.
128. Vo-Dinh, T: Biomediacl photonics:aA revolution at the interface of science and technology. In: Vo-Dinh T, editor. Biomedialc Photonics Handbook, Washington, DC:CRC Press; 2003. p. 1-1–1-18.
129. Zhang N, Contag PR, Purchio AF, et al: Tracking angiogenesis induced skin wounding and contact hypersensitivity using a VEGFR-2-luciferase transgenic mouse. Blood 2004;103:617-626.
130. Youngblood RC, Dickerson TW, Willard ST, et al: Methoxychlor exposure does not alter vascular endothelial growth factor receptor 2-mediated gene expression in a novel transgenic mouse wound model. FASEB 2004;Abstract # 787.5.
131. Regnault TRH, Orbus RJ, B. de Vrijer ML, et al: Placental expression of VEGF, OIGF and their receptors in a model of placental insufficiency-intrauterine growth restriction (PI-UGR). Placenta 2002;23:132-144.
132. Moulton K, Ryan P, Lay D, et al: Postmortem photonic imaging of *lux*-modified *Salmonella typhimurium* within the gastrointestinal tract of swine following oral inoculation *in vivo*. J Anim Sci 2009;87:2239-2244.
133. Moulton K, Ryan P, Christiansen D, et al: *Ex vivo* bioluminescence imaging of late gestation ewes following intra-uterine inoculation with and without *lux*-modified *Escherichia coli*. Comp Immunol Microbiol Infect 2009;32:429-438.
134. Moulton K, Ryan PL, Christiansen D, et al: Hormonal profiles of late gestation ewes following intra-uterine inoculation with and without *lux*-modified *Escherichia coli*. J Reprod Dev 2009;55:55-62.
135. Moulton K, Lovell F, Williams, E, et al: Use of glycerol as an optical clearing agent for enhancing photonic transference and detection of *Salmonella typhimurium* through porcine skin. J Biomed Optics 2006;11:1-8.

136. Curbelo J, Moulton K, Willard S. Photonic characteristics and ex vivo imaging of Escherichia coli-Xen14 within the bovine reproductive tract. Theriogenology 2010;73:48-55.
137. Ryan PL, Christiansen DL, Hopper RM, et al: Use of bioluminescence imaging technology and *lux*-modified bacteria to determine pathogen progression during uterine infections in the pregnant mare and rate of clearance post partum. Anim Reprod Sci 2010; http://dx.doi.org/10.1016/j.anireprosci.2010.04.051.

Common reproductive pathologies in reptiles

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Abstract

An overview of common reptile reproductive pathologies encountered in clinical practice is presented. Male and female reproductive tract infections and neoplasms are reviewed, as well as dystocia, egg yolk coelomitis, and hemipene impactions and prolapse. The information presented draws from the author's own cases as well as literature citations.

Keywords: Reptile, reproduction, pathology, neoplasia, infection, dystocia

Introduction

This paper will discuss of some of the more commonly encountered clinical problems involving the reproductive system in reptiles. In the author's practice the female reptile's reproductive system appears more prone to disease than that of the male. Pathology reviewed will include egg retention (dystocia), egg yolk coelomitis, bacterial infections (salpingitis, oophoritis, and hemipene/penis infection), hemipene impactions, hemipene prolapse, and neoplasia (of the ovaries, oviducts, and penis/hemipenes,).

Egg retention (egg binding, dystocias) in female reptiles

Female reptiles are frequently presented for egg retention, wherein ovarian follicles develop but the eggs are not laid within a reasonable period of time.[1-4] The normal interval between breeding/ovulation and egg-laying varies between species. Some reptile 'dystocias' are pre-ovulatory,[1,2] wherein the egg cycle is stalled during follicular development in the ovary, resulting in large follicles that fail to ovulate (follicular stasis). This is most common in lizards but has been documented in tortoises as well.[1,2,5] The follicles are not shelled which may make them more difficult to detect via palpation and radiography.

Some reptiles eventually complete the ovulatory cycle and lay shelled eggs; others may reabsorb the yolks and the developing eggs disappear. When neither of these events occurs within a reasonable time interval, then an abnormal ovulatory cycle may be suspected. Some reports indicate that reptiles without a breeding partner are more likely to have arrested follicular development.[6] The exact timeframe wherein a normal ovulation becomes problematic is not always clear; besides species differences, there is the tendency of many snakes and chelonians to hold follicles or mature eggs for some time without clinical signs of illness.[3] In general, reptiles that are active, alert and appear stable may be allowed some time to resolve a preovulatory dystocia. Barring successful resolution of the problem, ovariectomy or ovariosalpingectomy are viable treatment options.

Post-ovulatory dystocias are common in reptiles.[1-4] Once the eggs have ovulated and passed through the shell gland, they must be laid or removed surgically; reabsorption is no longer possible. In the author's experience, a post-ovulatory animal with shelled eggs present (as evidenced via visual inspection, palpation, radiography or ultrasound) usually undergoes oviposition within one to three weeks. Carrying the eggs for a more prolonged period of time is suggestive of abnormal egg retention.

Firm diagnosis of dystocia is often problematic. Females may demonstrate restlessness and digging behavior in their habitat (attempts to find a nesting site). Visible abdominal enlargement is often noted, especially in lizards. Egg binding often is accompanied by partial or total anorexia, either due to malaise or simply the distension of the coelomic cavity by the egg mass. It should be noted, however, that the above signs are also seen with normally gravid animals.[2,3]

True dystocias may be suspected when gravidity becomes unusually prolonged, or if other signs develop. Abdominal contractions and straining without successful egg-laying may be seen. Cloacal discharge or prolapse of cloacal tissues can occur. The affected animal may become lethargic and weak, a key sign that the reproductive process may be abnormal.[3] Producing only a portion of a clutch (with confirmation of retained eggs or young within the gravid animal) also suggests dystocia. Reptiles with the above signs usually require more aggressive treatment; those which have laid a partial clutch may warrant intervention within 48 hours.[3]

As a side note, viviparous animals such as boas and Jackson's chameleons (*Chamaeleo jacksoni*) may also retain their young, often the entire clutch. Many species have prolonged gestations, making determination of dystocia difficult. Telltale signs may include malaise, straining or attempts to pass young unsuccessfully, passage of a partial clutch, or cloacal discharge. Palpation is less useful at defining fetal masses than eggs; radiographs will visualize and allow counting of fetal skeletons. Ultrasound can identify fetuses and also assess their viability via detection of fetal heartbeats.[3]

Potential causes of dystocia are numerous,[1-4] but can be categorized into two main groups: behavioral and physiologic. Behavioral egg-binding may occur when a female reptile is inhibited from normal egg-laying behavior, due to stress or lack of a proper oviposition site. Many species excavate a pit or underground chamber in which eggs are deposited. Cage habitats often fail to accommodate for this behavior, and the animal may refuse to deposit eggs in an exposed location on the floor surface. Other potential stresses such as recent caging changes, noise and disturbances near the habitat should all be investigated as potential causes of dystocia.

Physiologic causes of egg retention include weak or absent oviduct contractions (oviduct inertia), congenital or acquired anatomic defects of the reproductive tract, trauma to eggs within the gravid female, and oversized or malformed eggs.[3,4] These physiologic dystocias may be further categorized as 'obstructive' or 'non-obstructive' to help determine appropriate treatment modalities.[3]

Oviduct inertia is often idiopathic, and behavioral egg retention is a differential diagnosis when no underlying physiologic cause can be identified. Environmental parameters should be carefully evaluated; air temperatures lower than the species' preferred range may reduce oviduct contraction and impair oviposition.[1,3] Temperature readings must be made with accurate thermometers placed in shielded locations (completely shaded from heat sources such as lamps) to assess ambient temperature. Nutritional imbalances or deficiencies have been implicated in many cases of reptile dystocias,[2,7] and a thorough dietary history should be taken. Low serum calcium levels, for instance, may reduce strength of oviductal contractions.[1,2]

Oviduct inertia may also be caused by disease such as bacterial oophoritis and salpingitis, or other illness unrelated to the reproductive tract.[3,7,8] A complete blood count (CBC) and serum chemistry evaluation may aid the clinician in detecting organic causes of oviduct inertia.

Defects or anomalies of the reproductive tract may also lead to inability to pass ova down the oviducts and through the cloaca.[3,7] Congenital or acquired strictures of the oviducts or lower reproductive tract are examples of disease conditions which may impair oviposition. Prior episodes of dystocia, especially those requiring surgical intervention, may increase the risk of stricture and repeated egg retention. Masses such as abscesses or neoplasms in the oviduct can also lead to dystocia.[3,4]

Anomalies of the eggs may also lead to dystocia. Occasional oversized eggs are produced, possibly due to presence of double yolks or other developmental defects. Fusion of two or more eggs is occasionally seen as well.[3,4,8] These anomalies of shape and/or size may inhibit the passage of ova down the reproductive tract.

Male turtles that breed aggressively have been reported to occasionally traumatize the eggs of gravid females; the penis may fracture one or more eggs, producing sharp shell fragments and oviduct lacerations. This can lead to life-threatening dystocia.[4]

Treatment of dystocia varies depending on the etiology and duration. Thorough history, physical examination, radiographs and ultrasonography are all useful tools in diagnosis and characterization of dystocias. Turtles with dystocia may be more difficult to diagnose on examination, due to the difficulty of palpating eggs within the shell. Digital palpation is sometimes possible via the inguinal (prefemoral) fossa or via the cloaca.[2,4] In addition turtle egg shells are more mineralized than those of most lizards and snakes, making radiographic visualization easier.[3]

Traumatized or malformed eggs may require surgical intervention, i.e. a celiotomy with salpingotomy or ovariosalpingectomy. Aspiration of oversized eggs, either percutaneously or via the cloaca, may also be performed to collapse egg(s) and allow passage via the cloaca.[2,3,8] This may be useful in turtles as an alternative to aggressive plastral celiotomy which requires cutting the shell. Surgical management of turtle dystocias can sometimes be accomplished via cutaneous incision cranial to the rear leg (the inguinal or pre-femoral fossa). This allows salpingotomy and egg removal but not ovariectomy.[2]

Oxytocin may resolve dystocia in some females with shelled eggs. This therapy is best utilized in patients lacking detectable egg or anatomic anomalies (i.e. non-obstructive dystocias), and who have not been egg-bound for a prolonged period.[3] Pretreatment with parenteral calcium is usually unnecessary unless low serum calcium levels or metabolic bone disease are documented;[4] when indicated, commonly cited doses of calcium gluconate range from 10-100 mg/kg intramuscularly or intracoelomic.[2,9-12] The response to oxytocin is variable, with some animals producing eggs within minutes, and others having no response at all. In general, the response to oxytocin is more consistent in chelonians than in lizards and snakes.[3] Placing the patient in a quiet covered enclosure, or back in the normal habitat with egg laying sites available, may facilitate oviposition.[4] Arginine vasotocin has also shown potential usefulness and efficacy in stimulating oviposition in reptiles.[13]

Even if eggs are not laid, the drug may help move the most caudally positioned egg down to the cloaca, where it can then be removed manually. Extraction may be facilitated via aspiration of the egg contents or manual fragmentation with forceps (if the egg is old and hardened). Once the most caudal egg is removed, the remaining eggs may pass without further assistance, or additional oxytocin injections may be used to move additional eggs

down to the cloaca where they can be manually removed one at a time. The author has achieved good results with this technique in some chelonians, and others have reported success as well.[2]

Published reptile doses for oxytocin are variable, but commonly range from 1-20 IU/kg.[3,4,14,15] The author gives up to three doses at 60 to 90 minute intervals, dosed incrementally at 2 IU/kg, 5 IU/kg, and 10 IU/kg if no effect is seen from the prior dose. Lack of response after three doses usually indicates that oxytocin will be ineffective. However, the author usually sends the patient home to observe in its familiar habitat overnight (with proper egg laying sites available). Some animals lay eggs within 24 hours of oxytocin administration once they are relaxed.

Manual manipulation of retained eggs toward the cloaca has been utilized by laypersons and veterinarians to resolve dystocia in snakes. Although this method can be effective when performed carefully, it entails significant risks including rupture of ova or oviducts.[3] Retained eggs may be adhered to the oviduct wall, and both ova and oviduct integrity may be compromised by disease, making the structures more fragile than in the normal animal. Thus manual manipulation of eggs cannot be recommended as a safe option in most dystocia cases.[3]

Egg yolk coelomitis in female reptiles

Lizards appear to be most prone to this condition, and most cases seen by the author have occurred in green iguanas (*Iguana iguana*) and leopard geckos (*Eublepharus macularius*). Bearded dragons (*Pogona vitticeps*) and chelonians have also been documented with yolk coelomitis.[2,3] The pathology most commonly involves females with large unshelled egg follicles which begin to leak yolk from the ovaries. In many cases the exact etiology is unproven, but a likely cause is trauma (such as falling) while gravid, which ruptures one or more follicles. The author has seen several cases wherein the owners recalled the animal falling while showing nesting behaviors, and then becoming lethargic and listless after the trauma.

Dystocia or urolithiasis may also lead to rupture of mature ova and damage to the oviduct, sometimes resulting in yolk leakage into the coelomic cavity.[16] Aggressive breeding attempts by male turtles may fracture eggs in gravid females, leading to yolk coelomitis in some cases.[4]

Bacterial salpingitis and oophoritis can lead to leakage of yolk into the coelomic cavity, and the author has seen yolk coelomitis secondary to oviduct adenocarcinoma in an iguana. The released yolk material incites a strong inflammatory 'foreign body' reaction in the coelomic cavity.[4,16] This can rapidly produce severe illness, with signs including malaise, lethargy, anorexia, and abdominal distension. A CBC and serum chemistries may reveal a leukocytosis with left shift; serum calcium levels may be elevated consistent with ovulation.[17] Egg follicles may be palpable, but the distension of the body cavity with yolk often precludes accurate palpation. Ultrasonography can verify presence of follicles or ova, and a simple abdominocentesis is often diagnostic: aspiration of the coelomic cavity typically yields small to copious amounts of bright yellow viscous fluid consistent with egg yolk. Microscopy reveals fat droplets and sometimes white blood cells in the yolk material.

Treatment involves celiotomy and removal of the egg follicles (typically via an ovariosalpingectomy).[3,4] The free yolk material in the coelomic cavity must be completely removed as well, or the severe inflammatory response will persist and can result in fatality.[4,16] Warmed saline lavages aid in removal of the material, which often adheres to the serosal surfaces. Reptiles lack a diaphragm, and the yolk material may pool anywhere in the coelomic cavity, including cranially in the thoracic area. Positive pressure ventilation to completely fill the lungs will aid in displacing the yolk material caudally into the abdominal region, where it can be readily removed. Although some authors in the past reported a high mortality rate with yolk coelomitis,[4] the author has achieved a high cure rate with early detection and aggressive treatment of this condition in iguanas, and other current literature has noted similar success.[3]

Bacterial infection of the reptile reproductive tract

Bacterial salpingitis (oviduct infection) or oophoritis (infection of ova) are occasionally seen in female reptiles.[2,4,7] In the author's experience this occurs most commonly when ova are present within the oviducts. The author has usually encountered salpingitis when a concurrent bacterial oophoritis was present; egg or follicle retention and degradation of yolk material may be factors predisposing to salpingitis.[8] Conversely, preexisting salpingitis may in theory predispose to oophoritis, and determining which condition preceded the other may be problematic.

Other factors which reduce immune competence, such as concurrent disease or husbandry problems, may increase risk of oviduct infection.[2] Definitive diagnosis may be difficult, as many cases lack a cloacal exudate. Detection of egg masses in the coelomic cavity (via palpation, radiography or ultrasonography), coupled with leukocytosis and signs of malaise, should increase the index of suspicion. Aspiration of the body cavity may yield an inflammatory exudate or free egg yolk.

The most effective treatment in the author's hands has been ovariosalpingectomy and broad spectrum antibiotic therapy. In valuable breeder animals, removal of infected ova via salpingotomy may be attempted. Oxytocin may induce oviposition, but can be ineffective even in healthy female reptiles.[3,4] With oophoritis or salpingitis, adhesions may form between the ova and the oviduct, and the integrity of the oviduct wall may also be compromised.[8] These factors make use of oxytocin in diseased animals more risky, as the oviduct may easily rupture, allowing dissemination of infection into the coelomic cavity. Surgery probably offers the highest success rate and safety margin in such patients.

In male reptiles, infection of the penis or hemipenes is occasionally encountered. Infection may be induced by trauma (especially during breeding),[8,18] sexually transmitted disease (such as mycobacterium),[8] or potentially by poor husbandry conditions causing immune suppression, such as low environmental temperature, dirty cage conditions, or dietary deficiencies. Hemipene impactions (see later discussion) may also predispose to infection in some lizard species.

Treatment includes debriding or amputating any necrotic tissue, manual cleaning and application of appropriate antibiotic topical preparations, and systemic antibiotics effective against gram negative aerobic bacteria (such as quinalones administered per os or IM). Culture and sensitivity testing of bacterial isolates from the lesion is ideal to guide selection of appropriate antibacterial drugs.

Hemipene impactions in lizards and snakes

Lizards such as green iguanas and leopard geckos are prone to impaction of the hemipene recesses with material consisting of hardened glandular secretions, keratinized cells, and sperm.[8,19] Impaction can cause swelling and distension of the hemipenes, and inability to extrude the organs when breeding. Similar impactions are also occasionally seen in snakes. The affected animal may be nonsymptomatic, or may demonstrate lethargy, straining, or swelling and eversion of the cloacal mucosa. The hemipene bulges in the ventral tail base may appear unusually prominent. The tips of the impacted hemipene material may be visible at the lateral aspects of the vent opening, and may look like traces of dried feces or necrotic tissue adhered to the mucosa. Primary or secondary infection of the impacted hemipene structures may be present.

This condition might be induced by factors such as low temperature or low environmental humidity, which may contribute to retention and hardening of the normal glandular secretions. In the author's experience impactions occur most commonly in reptile species with moderate to high humidity requirements, suggesting that dry cage conditions may be a factor.

Treatment is via gentle extraction (with forceps and digital massage) of the impacted material, and treatment with broad spectrum antibiotics (such as quinalones) if the tissues appear severely inflamed or infected. Resolution of existing husbandry problems is paramount as well.

Prolapse of the hemipenes/penis

Occasional prolapse of a hemipene (in snakes and lizards) or penis (in turtles) occurs. Underlying causes can include trauma to the organ, infection, hemipene impaction, straining while constipated, neurologic deficits to the retractor penis muscles or cloacal sphincter, neoplasia, and other factors.[4,19,20] Breeding-related traumas are not uncommon.[8,18] Husbandry problems such as cool environmental temperatures predispose to both sepsis and straining to eliminate wastes, which may promote penile prolapse. If caught early, the prolapsed organ may be cleaned and replaced *in situ*. The everted tissues, once cleaned, can be lubricated with an antibacterial cream such as silver sulfadiazine prior to reducing the prolapse. The vent may need to be sutured partially closed to prevent immediate re-prolapsing; provided that the patient can eliminate wastes, the sutures may be left in for weeks or even months with negligible negative effects.

In many cases the prolapsed penile tissue is traumatized, dry and necrotic, and must be amputated. With lizard or snake hemipenes this is easily accomplished with absorbable suture ligature of the base of the organ, followed by scalpel excision of the necrotic portion. In these cases the contralateral hemipene may still be viable and the animals can breed successfully in the future. The turtle penis is vascular and often more substantial in thickness; the blood supply and corpus cavernosum on each side should be ligated separately with transfixing horizontal mattress pattern absorbable suture.[21] The reptile penis does not contain a urethra, and amputation does not interfere with urination. Antibiotics should be used to prevent secondary infection (or to control preexisting infection); broad spectrum drugs are indicated to cover many of the enteric bacteria as well as surface opportunists. Good postoperative husbandry, as always, is essential.

Neoplasia of the reptile reproductive system

Neoplasia in reptiles is occasionally encountered in older individuals, especially in the female reproductive system. Literature reports are sparse compared to the data compiled for many common mammalian species. To date various ovarian and oviduct neoplasms have been reported. Turtles have been diagnosed with ovarian dysgerminomas,[4,22] oviductal leiomyosarcoma and cloacal polyposes.[22] Ovarian adenocarcinoma and ovarian teratoma have been seen in lizards,[23,24] and various ovarian and oviductal tumors have been documented in snakes, including hemangiomas, granulosa cell tumors, fibromas, leiomyosarcoma, tubular adenoma, and carcinomas.[4,23,25-28] The author has seen oviductal adenocarcinoma in a four-year-old green iguana (*Iguana iguana*) and a very large ovarian adenocarcinoma in a 12-year-old boa constrictor (*Boa constrictor*) with metastasis to the mesentery.

Affected individuals may exhibit nonspecific lethargy and malaise, loss of appetite, and gradual weight loss. Some individuals have detectable abdominal distension or palpable masses in the coelomic cavity. Radiographs, ultrasound, needle aspiration of masses and exploratory surgery may confirm the presence of neoplasia. Total excision of the masses, when possible, may be curative. Chemotherapeutic regimens in reptiles are in need of further study but may be attempted.[29] Metastatic neoplasms carry a poor long term prognosis. Surgery may nonetheless improve quality of life for an extended period in some cases; the boa ovarian adenocarcinoma treated by the author had visibly metastasized, but the owner reported the snake doing well two years postoperatively.

Occasional neoplasia involving the testes, penis or hemipenes may be seen in male reptiles. Reported testicular neoplasms include interstitial cell tumor in the desert tortoise (*Xerbates agassizi*),[4] and also in the Komodo dragon (*Varanus komodoensis*).[30] Seminoma was documented in an American alligator (*Alligator mississippiensis*).[31] Snakes have been diagnosed with seminoma, Sertoli cell tumor, and interstitial cell tumor.[4,23,27]

The author has seen one case of squamous cell carcinoma involving a hemipene of a 4.5-year-old green iguana. Such neoplasms may be aggressive and invasive, and attempted excision (via penile amputation) failed to cure the author's patient. Other treatment modalities such as chemotherapeutics or radiation therapy may be attempted when appropriate to the type of neoplasm.

References

1. Funk RS: Lizard reproductive medicine and surgery. Vet Clin North Am Exotic Anim Pract 2002;5:579-613.
2. Innis CJ, Boyer TH: Chelonian reproductive disorders. Vet Clin North Am Exotic Anim Pract 2002;5:555-78.
3. DeNardo D: Dystocias. In: Mader DR, editor. Reptile medicine and surgery, 2nd ed. Philadelphia: WB Saunders; 2006. p. 787-792.
4. Frey FL: Biomedical and surgical aspects of captive reptile husbandry, 2nd ed. Malabar, (FL): Krieger; 1991. p. 373-377,581,589.
5. McArthur S: Follicular stasis in captive chelonia, *Testudo* spp. Proc Assoc Reptil Amphib Vet 2001; p. 75-86.
6. Denardo DF, Autumn K: Effect of male presence on reproductive activity in captive female blood pythons, *Python curtus.* Copeia 2001;4:1138-1141.
7. DeNardo D: Reproductive biology. In: Mader DR, editor. Reptile medicine and surgery. Philadelphia: WB Saunders; 1996. p. 212-224.
8. Stahl SJ: Veterinary management of snake reproduction. Vet Clin North Am Exotic Anim Pract 2002;5:616-636.
9. Jenkins JR: A formulary for reptile and amphibian medicine. Proc Annu Avian/Exotic Anim Med Symp 1991.
10. Bauck L, Boyer TH, Brown SA, et al: Exotic animal formulary. Lakewood (CO): American Animal Hospital Association; 1995.
11. Stein G: Reptile and amphibian formulary. In: Mader DR, editor. Reptile medicine and surgery. Philadelphia: WB Saunders; 1996.
12. Scott BW: Nutritional diseases. In: Beynon PH, Lawton MPC, Cooper JE, editors. Manual of reptiles. Ames: Iowa State University Press; 1992.
13. Lloyd ML: Reptilian dystocias review--causes, prevention, management and comments on the synthetic hormone vasotocin. Proc Annu Conf Amer Assoc Zoo Vet 1990; p. 290-296.
14. Jenkins JR: Medical management of reptile patients. Compend Cont Educ Pract Vet 1991;13:980-988.
15. Gauvin J: Drug therapy in reptiles. Semin Avian Exotic Pet Med 1993;2:48-59.
16. Mader DR, Bennett RA, Funk RS, et al: Surgery: soft tissue, orthopedics, and fracture repair. In: Mader DR, editor. Reptile medicine and surgery, 2nd ed. Philadelphia: WB Saunders; 2006. p. 595.
17. Denardo D: Reproductive biology. In: Mader DR, editor. Reptile medicine and surgery, 2nd ed. Philadelphia: WB Saunders; 2006. p. 389.
18. Lock, BA: Reproductive surgery in reptiles. Vet Clin North Am Exotic Anim Prac 2000;3:733-752.
19. Barten, SL: Penile prolapse. In: Mader DR, editor. Reptile medicine and surgery, 2nd ed. Philadelphia: WB Saunders; 2006. p. 862-864.
20. Zwart P: Urogenital system. In: Benyon PH, Lawton MPC, Cooper JE, editors. Manual of reptiles. Gloucestershire: British Small Animal Veterinary Association; 1992.
21. Millichamp NJ: Surgical techniques in reptiles. In: Jacobsen ER, Kollias GV, editors. Contemporary issues in small animal practice: exotic animals. New York: Churchill Livingstone; 1988.
22. Frye FL:The diagnosis and surgical treatment of reptilian neoplasms with a compilation of cases 1966-1993. In Vivo 1994;8:885-892.
23. Harshbarger JC: Activities registry of tumors in lower animals, 1965-1973. Washington (DC): Smithsonian Institute Press; 1974.
24. Tocidiowski ME, Merrill CL, Loomis MR, et al: Teratoma in desert grassland whiptail lizards (*Cnemidophorus uniparens*). J Zoo Anim Med 2001;32:257.

25. Effron M, Griner L, Berirschke K: Nature and rate of neoplasia in captive wild mammals, birds and reptiles at necropsy. J Nat Cancer Inst 1977; 591.
26. Michaels SJ, Sanecki R: Undifferentiated carcinoma in the ovary of a boa constrictor (*Boa constrictor ortoni*). J Zoo Anim Med 1988;19:237.
27. Ramsay EC, Fowler M: Reptile neoplasms at the Sacramento zoo, 1981-1991. Proc Joint Conf Am Assn Zoo Vet Am Assoc Wildl Vet 1992.
28. Harshbarger JC: Activities report registry of tumors in lower animals, 1978 supplement. Washington (DC): Smithsonian Institute Press;1979.
29. Mauldin GN, Done LB: Oncology. In: Mader DR, editor. Reptile medicine and surgery, 2nd ed. Philadelphia: WB Saunders; 2006. p.317-20.
30. Harshbarger JC: Activities report registry of tumors in lower animals, 1975 supplement. Washington (DC): Smithsonian Institute Press;1976.
31. Wadsworth JR, Hill WCO: Selected tumors from the London zoo menagerie. Univ Pa Vet Ext Quart 1956; 141:170.

Ovarian disease in the dog: perspectives and treatment options

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Abstract

This overview includes common functional disorders of the canine ovary including hypoluteoidism, anovulation, aging change, oophoritis and ovarian remnant syndrome. Ovarian and periovarian cysts are described with an emphasis on clinical significance. Finally, neoplastic disease including epithelial, sex cord stromal and germ cell tumors of the ovary are outlined. The pathophysiology, anamnesis, physical examination findings, diagnostic techniques and methods of management are discussed.

Keywords: Ovary, ovarian function, neoplasia, cysts, canine

Introduction

This overview includes functional ovarian diseases including inadequate progesterone production during pregnancy, ovulatory failure, erratic interestrous intervals in aged bitches and resumption of estrus in neutered dogs. Lesions of the ovary detected by ultrasonography or at surgery are also described, including cystic ovarian and periovarian structures, and variation in ovarian size including neoplasia and oophoritis. We will concentrate on those that are common or clinically significant.

Clinical functional conditions

Hypoluteoidism

Hypoluteoidism is failure of maintenance of luteal function for the normal timeframe following a normal luteinizing hormone (LH) surge during estrus. During a normal, non-pregnant diestrus period, progesterone production from the corpus luteum (CL) may last from 42–75 days after the LH surge. Hypoluteoidism results in a shortened diestrual period and may result in a decreased interestrous interval.[1] During pregnancy, progesterone is produced for 65 +/- 1 day after the LH surge. Hypoluteoidism may result in resorption, abortion and delivery of pre-term fetuses.[1-5] There is variation in progesterone concentrations during the estrous cycle of the bitch and a definitive diagnosis of hypoluteoidism is difficult unless pregnancy failure has occurred.[1] Bitches with hypoluteoidism may habitually abort at the same stage of pregnancy. A progesterone concentration of at least 2 ng/ml is necessary for maintenance of pregnancy; a progesterone concentration below this level prior to day 42 of diestrus or day 61 of pregnancy, confirms the diagnosis.[1,3,5,6]

Corpora lutea produce progesterone for the entire length of pregnancy. During the second half of pregnancy, prolactin and LH provide additional luteotrophic support to maintain the CL.[1-3] Causes of hypoluteoidism include 1) abnormal CL function; 2) early luteolysis due to prostaglandin F2α (PGF) release from the endometrium as a result of inflammation or infection; 3) iatrogenic luteolysis; or 4) idiopathic reasons.[1] Where there is abnormal CL function, hypoluteoidism may occur at any stage of diestrus or pregnancy but most commonly occurs at mid-pregnancy or mid-diestrus when progesterone levels should be stable or just starting to decrease, and when support from relaxin and LH should occur but does not happen normally.[1-2] Hypoluteoidism resulting from inflammation or infection typically occurs later in pregnancy or diestrus.

Treatment of hypoluteoidism during pregnancy consists of supplementation of progesterone +/- use of tocolytics, anti-inflammatory medications and antibiotics.[2,4-6] Progesterone may be supplemented in one of four forms: 1) progesterone in oil (2 mg/kg i.m. every 2–3 days); 2) altrenogest (Regumate®, Intervet/Schering Plough, Millsboro, DE, 0.088 mg/kg p.o. q.d.); 3) micronized progesterone (Prometrium®, Abbott Laboratories, Abbott Park, IL, 10 mg/kg p.o. q.d.); and 4) medroxyprogesterone acetate (MPA) (0.1 mg/kg p.o q.d.).[1,3,5] Use of progesterone in oil or micronized progesterone prevents monitoring the endogenous progesterone concentration of the bitch, while use of altrenogest or MPA does not. With either progesterone in oil or micronized progesterone, dosage is adjusted after measurement of progesterone concentration every two or three days so as to determine a 'normal' progesterone profile for that pregnancy. The minimum dose of the synthetic progestagen, altrenogest, to maintain pregnancy is not known for each individual bitch. Tapering of the dose during the last 7 -10 days of pregnancy, first by one-fourth and then by one-half can usually be safely performed but otherwise bitches must be maintained at the dose listed. Caution must be used when supplementing progesterone as any intrauterine infection will be exacerbated and pyometra could occur. Serial ultrasound examinations and complete blood counts are required to detect this complication early and before systemic illness.[1-2,4]

Tocodynometry to monitor uterine muscular activity allows a minimal dose of progesterone to be used while still maintaining a quiescent uterus.[6] The addition of tocolytics (i.e. terbutaline starting at a dose of 0.03 mg/kg p.o. t.i.d. and adjusting according to response), will also decrease the progesterone dose required to prevent uterine contractions.[6]

Complications of progesterone supplementation include decreased uterine tone and subsequent dystocia, decreased mothering instinct, and decreased mammary development and lactation.[2-3] Decreased mammary development can be prevented through use of the dopamine antagonists, metoclopramide or domperidone.

When hypoluteoidism is suspect, progesterone monitoring should begin at 25–30 days gestational age (usually at the time of the pregnancy ultrasound) and repeat testing performed at appropriate intervals depending on the progesterone level. Initiation of progesterone supplementation should be delayed as long as possible to reduce the risk of masculinization of female fetuses or exacerbating intrauterine infection.[2] Use of progestagens in human pregnancy is implicated in causing congenital heart defects, limb-reduction deformities, and hypospadias in male fetuses.[7]

In non-pregnant bitches where a decreased interestrous interval occurs, ovulation should be documented and then progesterone monitored post-ovulation. If progesterone concentrations reach baseline levels (<2 ng/ml) prior to day 40, luteal insufficiency should be suspect and an underlying cause investigated. If an underlying cause is detected it should be treated. If not, an appropriate interestrous interval should be created using mibolerone to suppress estrus and then when the bitch is bred, she should be monitored carefully for luteal insufficiency during the pregnancy.

Anovulatory cycles

Ovulatory failure occurs when oocytes are not released from follicles. Corpora lutea then do not develop. Persistent estrus or prolonged proestrus typically results and estrogen secretion is prolonged. Ovulatory failure is reported in 1.2% of bitches presented for breeding management. This condition may be hereditary.[4]

The functional defect may be caused by failure of release of gonadotropin releasing hormone (GnRH) or LH from the hypothalamus or pituitary, respectively; failure of LH to bind to receptors on the follicle; failure of follicles to produce adequate estrogen to stimulate GnRH surge; immune mediated oophoritis; cachexia or obesity; stress (travel, performance, kennel); adrenal disease (hypo- or hyperadrenocorticism); or chromosomal abnormalities.[8] Follicular cysts and estrogen producing neoplasia may mimic an anovulatory cycle.

Signs of an anovulatory cycle include prolonged proestrus or estrus; vulvar edema; sanguinous vulvar discharge; and nymphomania. The bitch may appear to progress through proestrus to estrus, but does not ovulate. Eventually, in most cases, the bitch will slip back into proestrus or anestrus. Vaginal cytology and speculum examination may reveal either a blotchy pink mucosa without edema and no signs of metestrual cells or foam cells (typical of diestrus) or a light pink and edematous mucosa and a typical early proestrus cytologic appearance.[8] In some cases, a split heat will ensue with ovulation occurring during the second proestrus period. If no ovulation occurs there will be no diestrual period, and a decreased interestrous interval may result.[4]

Diagnosis is made by the lack of rise in progesterone concentration as normally occurs with ovulation. The normal progesterone concentration at the time of ovulation (4-10 ng/ml) rises at least 3-4 ng/ml a day culminating in a peak progesterone concentration of 20 ng/ml or more (it may reach 80+ ng/ml in some individuals). During an anovulatory cycle, progesterone will usually be in the range of 3-8 ng/ml but rarely exceeds 10 ng/ml. If it does exceed 10 ng/ml, it falls toward the baseline value within a few days.[8] Serial ultrasonography is required to confirm a lack of ovulation.[9-10] Ovulatory changes are very subtle and difficult to determine if scans are not compared daily.

Treatment includes hormonal induction of ovulation or luteinization of follicles.[4] Ovulation induction should be attempted when vaginal cytology indicates maximal estrogen secretion (>70% anucleated superficial cells) and follicles attain pre-ovulatory size (4-5 mm toy and small breeds; 5-7 mm medium-large breeds; 7-10 mm giant breeds). Ovulation may be induced with GnRH (1.1-2.2 µg/kg i.m. or i.v.; repeated daily for 1-3 days if necessary); human chorionic gonadotropib (hCG; 500-1000 IU/bitch i.m.; repeated in 2-3 days if ovulation does not occur); a combination of hCG and GnRH; or deslorelin implant (should be removed once ovulation is documented). Ovulation can be induced in up to 50% of treated cases. Progesterone concentration should be monitored throughout pregnancy as hypoluteoidism may develop.

Age related changes

As bitches age, ovarian function may become erratic. Interestrous intervals can be abbreviated or prolonged, anovulatory cycles may increase in frequency, and follicular cysts may develop. Ovarian senescence usually never results in complete cessation of function.[3,11] Ovarian cysts are more common in aging bitches (see below).

Ovarian remnant syndrome

Ovarian remnant syndrome occurs when estrus activity begins or continues after ovariectomy or ovariohysterectomy.[3,12-14] With this condition, all or part of the ovary remains either in the location of the normal ovary or elsewhere in the abdomen if it is transplanted intentionally or accidently. The remaining tissue becomes active again following revascularization.[3,12-14] There are rare cases where a supernumerary ovary or accessory ovarian tissue is present. The right ovary is more likely to be incompletely removed as it is more cranial in location and is more difficult to remove when surgery is done with a small incision.[14]

Return of cyclic behavior after surgery may begin either at a normal interestrous interval or months to years later.[3,12,13,15] The clinical signs will vary depending on the amount of ovary and uterus that remains. Vulvar edema will develop and male interest and receptivity will occur. If part of the uterine body remains, a bloody vulvar discharge will be present. If a uterine stump remains, pyometra, mucometra, hydrometra or hematometra may develop. In some cases, neoplastic transformation will occur in the ovarian remnant (see ovarian neoplasia below).[12,16]

Diagnosis is made by a vaginal cytology suggestive of proestrus, estrus or diestrus. Progesterone concentration will rise after ovulation. Ovulation can be induced at estrus by administration of hCG (500-1000 IU i.m.) or GnRH (1.1-2.3 μg/kg i.m.or i.v.) and then confirmed by measuring progesterone concentration five to seven 7 days later. If progesterone is elevated, ovulation has occurred. Ultrasonography can sometimes be used to locate the remnant if it is large enough to visualize. Surgical removal is recommended after ovulation has occurred or been induced since a remnant enlarged with a luteal tissue is easier to find than one with follicular structures.[3,12-15]

Cysts of the ovary and surrounds

Cysts in and near the ovary may be functionally significant, represent neoplastic transformation of ovarian structures or are incidental and represent differential diagnoses for significant lesions. The cysts are best divided into those that are within the ovary and those that are outside the ovary.

Intraovarian cysts

Cysts arising within the ovary include those that are functionally significant (follicular cyts and luteinized cysts) and those that are rarely significant (subsurface epithelial structures and cystic rete ovarii) or those that are not significant and are incidental lesions (cystic CL). Neoplasms of the ovary can be cystic in appearance and are described below.

Follicular cysts

A follicular cyst resembles a Graafian follicle that is larger than a normal follicle. This is generally believed to be any follicle over 7 mm diameter.[3] They either are identical to follicles with a lining of granulosa cells, a single flattened layer or a sclerotic wall.[17,18] They may be single or multiple, and unilateral or bilateral. The largest follicular cyst reported exceeded 30 cm![3]

It is believed that follicular cysts arise when the hypothalamic-hypophyseal-ovarian (HHO) axis is abnormal, either because of a failure of LH release or a lack of response of a Graafian follicle to LH.

Those follicular cysts that are endocrinologically active produce estrogens.[3,4,6,19] Persistent estrus or nymphomania and eventually estrogen toxicosis can occur.[3,4,6,19] Prolonged proestrus or estrus is commonly seen. Estrogen concentrations fluctuate and bitches in estrus may return to proestrus or anestrus repeatedly. Vaginal cytology of affected dogs will show a progression from proestrus to estrus but no evidence of diestrus unless ovulation or luteinization of the structures occur.[15,20] On ovarian ultrasonography large (>7mm) anechoic structures with a thin wall are seen.[3,9-10] Signs of estrogen toxicosis include vulvar edema, gynecomastia, bloody vulvar discharge, and endocrine skin disease with ventral alopecia and hyperpigmentation, lichenification, miliary sebaceous cysts, seborrhea, ceruminous otitis, and pruritus.[3,21] Hyperestrogenism may also result in bone marrow failure and pancytopenia.[3,22] The cyst(s) may be found at the time of pregnancy ultrasonography. In some cases, pregnancy failure occurs and the total estrogen concentration is increased above normal for diestrus or pregnancy. Embryo transportation in the uterine tube or uterus may be affected.

Treatment involves administration of a gonadotropin (hCG, GnRH, deslorelin) to luteinize the cyst(s).[4,6,15,20] Luteal function will not last more than a few weeks and progesterone concentrations typically do not exceed 10 ng/ml, indicating abnormal luteal function. Bitches with follicular cysts may have a reoccurrence on subsequent cycles. If a normal diestrual period does not follow ovulation, a shortened interestrous interval may occur.[15,23]

Luteinized cysts

A luteinized cyst forms when a follicular cyst luteinizes. They are larger than normal follicles and have a thin layer of luteal cells at the periphery. They are uncommon.[17-18,24] They typically produce low concentrations of progesterone for a prolonged period of time, often well beyond an expected diestrual period.[3-4,6,15] Diagnosis is made by measuring elevated serial progesterone concentrations (more than 72 days > 2ng/ml) and confirmed with ultrasonography.[3-4,6,9,10] Treatment includes serial PGF administration. If unsuccessful, surgical removal of the cyst or unilateral ovariectomy is necessary.[4,6,15,23]

Cystic corpora lutea

A cystic corpus luteum forms when a CL fails to completely luteinize.[17] All corpora lutea in dogs initially have a cystic center that eventually fills. All CL will appear cystic on ultrasonography immediately after ovulation. As the luteal phase progresses, the center of the CL will become more hyperechoic in most cases, but this can take days to weeks to visualize.[9,10] Cystic CL have no clinical significance.

Cystic rete ovarii

The rete ovarii are remnants of embryonic rete tubules and are within the medulla of the ovary.[23,25] They communicate with mesonephric tubules that are similar in appearance but have a smooth muscle wall. As with mesonephric tubules, these may dilate.[18] Dilation is common and occurs in about 10% of dogs.[26] They are usually multiple, variably sized and up to several centimeters in diameter. When large, they can compress the ovarian cortex[3]

These cysts are generally found incidentally on ultrasonography, at the time of ovariohysterectomy (OHE) or at necropsy. There are no clinical signs with these cysts as they do not produce hormones. If they become large enough to compress the ovarian stroma they could prevent or disrupt ovulation.[23,25] They can become hyperplasic and neoplastic (see below).

Cystic subsurface epithelial structures

Cysts of the normally occurring subsurface epithelial structures (SES) are common. They are similar to epithelial inclusion cysts of other species - except that the dog normally has 'downgrowths' of the surface epithelium. They are not seen in young bitches but occur with increasing frequency in older dogs. They are seldom larger than 5 mm and they are lined by a single layer of cuboidal cells.[3,17,18,23,25] They are a common site of development of adenomas and carcinomas (see below). Unless they develop into neoplastic tissue, these cysts are benign and are found incidentally on ultrasonography, at the time of OHE or at necropsy.

Extraovarian (periovarian) cysts

There are three basic types of cysts around the ovary–they derive from the embryonic remains of the paramesonephric duct, mesonephric duct and mesonephric tubule. Location is the best guide, but histological features may help differentiate these three. Cysts of the paramesonephric duct are cranial to the ovary and within the mesovarium. They have ciliated and non-ciliated epithelium, no basement membrane and a thin layer of smooth muscle. Cysts of the mesonephric duct are parallel to the structures derived from the paramesonephric duct, are non-ciliated, have a basement membrane and a thick layer of smooth muscle. Cysts of the mesonephric tubules are between the ovary and the fimbria (cranial to the ovary), may be lined by ciliated cells and have basement membrane and a thin smooth muscle layer.[18]

None of these cystic structures are clinically significant. They are found incidentally at ultrasonography, OHE or laparotomy, or at necropsy. No treatment is required.[3]

Variation in ovarian size

The important variations in ovarian size are ovarian hypoplasia and aplasia, and neoplasia.

Ovarian hypoplasia or aplasia

Complete ovarian aplasia is extremely rare. Gonadal dysgenesis and ovarian hypoplasia is seen with disorders of sexual development (DSD) and especially with chromosomal abnormalities.[3,6,27-31] Affected bitches present with prolonged anestrus or failure to cycle.[3,27] There are many DSD where the ovaries are hypoplastic.[3,27,28,30,31] Diagnosis is based on history, physical examination, ultrasonography revealing small or absent ovarian tissue, and a documented failure to cycle over a period of more than a year after puberty should have occurred. Complete assessment of a DSD requires karyotyping, ovarian histology, hormone profiling and documentation of the internal and external genitalia.

Ovarian neoplasia

Neoplasms of the ovary resemble any of the normal ovarian structures. The ovary begins embryologically as mesenchyme and mesothelium of the gonadal ridge, and is populated with 'germ' cells, and develops epithelial and stromal tissues, rete ovarii, endocrine tissue, blood vessels, and many other cell phenotypes. Neoplasms have traditionally been divided into groups based on phenotype, and now immunohistochemistry assists us, especially in neoplasms that are anaplastic. The basic divisions are into epithelial, sex cord-stromal, and germ cell types. Mixed tumors of the ovary are very rare.

Clinically affected bitches have a prolonged anestrus, persistent estrus, or have no cycle abnormalities.[3,4,6,28,32] Neoplastic transformation can occur in bitches with ovarian remnant syndrome.[16,32] Ovarian neoplasia may be diagnosed 1) via abdominal palpation of a mass in the mid-abdomen; 2) incidentally at laparotomy or during OHE; 3) as a result of endocrine changes from hormones produced by the tumor; 4) due to effects of metastasis to other organs,[3,6] 5) on ultrasonography of the abdomen; or 6) as a soft tissue density in the area of the kidney on abdominal radiographs.[9]

Epithelial neoplasia of ovary

Epithelial tissues of the ovary includes the surface 'germinal epithelium', which is modified mesothelium, subsurface epithelial structures derived from the surface epithelium, and the rete ovarii derived from mesonephric tubules and/or cords.[23] Many tumors arise in SES and are often multifocal. Others appear to only involve the germinal epithelium. According to the World Health Organization publication, *Tumors of the Genital System*, the types of epithelial neoplasms of the ovary are 1) papillary adenoma and cystadenoma, 2) papillary adenocarcinoma and 3) rete adenoma.[33] Papillary adenomas are smooth and nodular or papillary and are within distended subsurface epithelial structures. They are often multiple and are composed of small cuboidal or cylindrical cells that may have cilia. Mitoses are rare. They may have a glandular or cystic appearance. Papillary carcinomas have a shaggy appearance, increased mitotic activity, invasion into the ovarian stroma and extension into the ovarian bursa. Most important is the statement 'distinguishing between them can be difficult'. The separation of epithelial tumors into 'benign' and 'malignant' phenotypes is therefore subjective. Epithelial tumors of the surface or SES progress from papillary hyperplasia to adenoma to carcinoma, and all three forms can sometimes be identified in the same ovary. Subcategorization of epithelial tumors is based on their histological appearance, and not on their clinical behavior. A detailed long term follow-up of epithelial tumors is needed to accurately predict outcome for individual cases.

There is no hormone production with these tumors, so they will be diagnosed on physical examination, ultrasonography, abdominal radiography, exploratory surgery or at necropsy.[3] The most common clinical signs, when present, are abdominal distension and ascites.[3]

Adenoma

Adenoma implies a benign and well differentiated phenotype, lack of aggressive features such as invasion, and a low mitotic rate. The so-called papillary adenomas are located at the periphery of the ovary, and often in the SES. Adenomas of the rete ovarii are extremely rare. Adenomas of the SES are often multiple, vary in size from microscopic to macroscopic and should not metastasize. There is an apparent gradation from hyperplasia to adenoma. The term cystadenoma is also used but most adenomas are a mixture of this and other phenotypes including solid tumors. They appear to be unilateral tumors and are the most common.[17,34-36] There is no hormone production by these tumors, so they will be diagnosed on physical examination, ultrasonography, abdominal radiography, exploratory surgery or at necropsy.

Ovarian carcinoma

Carcinomas have aggressive features such as invasion of the stroma, increased mitosis, and spread to the bursal tissues and abdomen. Studies indicate that these can be bilateral, and about one-half have metastases, usually throughout the peritoneum.[34,36,37] They arise from the surface of the ovary or the SES. Rete carcinomas are not reported.

There is no hormone production by these tumors, so they will be diagnosed on physical examination, ultrasonography, abdominal radiography, exploratory surgery or at necropsy. Signs typically result from abdominal distension or effusion, weakness, lethargy, or (multi)organ specific dysfunction.

Sex cord-stromal neoplasms (ScSN)

The sex cord stromal tumors of the ovary include those that resemble the cells derived from the sex cords including granulosa cells, theca cells, luteal cells, and interstitial endocrine cells, but separate from those tumors such as pure fibromas and leiomyomas that occur in any location in the body.[23] They include the most well known neoplasm of them all - the granulosa cell tumor (GCT), as well as neoplasms with cells that resemble cells of the theca, and the corpus luteum. Some resemble the Sertoli cell tumor of the testis, and the interstitial cells of the testis.[23] Many have multiple patterns, so separating the neoplasms into groups is artificial and from the knowledge available, has little prognostic relevance. It would be a perfect world if each neoplasm were a pure representative of its type and this was related to a specific prognosis or hormonal profile. The most common type is reported to be the GCT, but most also have cells resembling the thecal layers and are called granulosa-theca cell tumors. Comparing studies where different criteria are used in classification is impossible. The list includes ScSN –GCT, ScSN – thecoma, ScSN – luteoma, ScSN – Sertoli cell tumor, ScSN – interstitial cell tumor, ScSN with granulosa cell and thecal differentiation. The most common is the ScSN with either complete or predominantly granulosa cell differentiation. All of the others are rare and will not be described here.

This group of neoplasms has the potential to secrete hormones. The most common hormone produced is estrogen, but testosterone or low levels of progesterone may also be produced.[3,32,38] Clinical signs are attributable to the type of hormone being produced. If estrogen is being produced then prolonged estrus behavior, nymphomania, and estrogen toxicity may occur (see follicular cysts above). If progesterone is being produced then prolonged anestrous, cystic endometrial hyperplasia, mucometra or pyometra may occur. If testosterone is being produced then male behaviors (deepening of the voice, mounting, marking, aggression) and peri-anal neoplasia may develop.

ScSN granulosa cell (GCT) and granulosa-theca cell tumors

The granulosa cells derive from the follicular cells that surround the oocyte, and probably arise from the sex cords.[17] Around the granulosa cells are the thecal cells, which resemble fibrocytes. The reports on GCTs vary greatly. It is clear that some people classified most sex cord-stroma tumors as GCTs, and others 'split' them into distinct (but also overlapping) categories including granulosa-theca tumors.

Macroscopically, these have a nodular surface and are surrounded by ovarian bursa. There are often regions of interstitial hemorrhage. The cut surface is cystic with white solid areas. Histologically they had broad fibrous septae, then finer septae that divided the tumors into multiple solid nodules of cells that resembled granulosa cells. Formation of cysts lined by cells resembling the inner lining of follicles happens to a greater or lesser amount.

Bitches with GCT may present with prolonged anestrus or persistent proestrus/estrus.[3,4,32,38-40] Bitches of all age groups may develop GCT and they may develop in an ovarian remnant.[32,39] If hyperestrogenism is prolonged, estrogen toxicosis can develop.[32,38] These tumors may be large enough to be palpated through the abdominal wall. They may be seen on abdominal radiographs as a soft tissue density in the area of the kidney. Ultrasonographic appearance may be solid to cystic.[9] The contralateral ovary is usually inactive and very small. They are rarely bilateral. Return to normal estrus can take weeks to years depending on the degree of suppression to the HHO axis.

The metastatic rate is reported to be from 5 to 30% .[35,37] When metastasis occurs, signs may be attributable to the organ(s) of metastasis. Metastasis can be to virtually any organ of the body.[39-41]

Germ cell tumors of the ovary (including teratoma)

Dysgerminoma. Dysgerminoma is one of the most common ovarian germ cell tumors. They are the ovarian equivalent of the testicular seminoma, so they have a smooth but nodular outer surface and vary from white grey to dark red (with hemorrhage and necrosis). Microscopically they are round cell tumors with cells forming diffuse sheets. Most cells are uniform but some have large single nuclei and others are multinucleate. Foci of lymphocytes are sometimes seen[37,42] Rare examples are metastatic.[37,42-44] There is no hormone production by these tumors, so they will be diagnosed on physical examination, ultrasonography, abdominal radiography, exploratory surgery or at necropsy.[3]

Teratoma. Teratomas have several of the three germ cell layers - ectoderm, mesoderm and endoderm - all within the same tumor. They are usually smooth and multinodular, and have cyst-like structures containing hair, a greasy gelatinous material and solid areas with grey-white areas (bone), translucent blue (cartilage) and black foci. Histologically they contain epidermis, hair, respiratory epithelium, cartilage, bone, fat, collagen and brain and nerves.[45-46] Less than 50% are metastatic.[42] There is usually no hormone production by these tumors, so they will be diagnosed on physical examination, ultrasonography, abdominal radiography, exploratory surgery or at necropsy.[3]

Inflammation of the ovary

Oophoritis is rare in the bitch. Reported cases of suspected immune mediated disease have diffuse inflammation dominated by lymphocytes and plasma cells, and degeneration of oocytes.[3,27,28,47] Oophoritis can occur following *Brucella canis* infection, after sepsis or in association with local inflammation. Persistent estrus with ovulation failure and/or hypoluteoidism may occur as a result or animals may present with prolonged anestrous.[3,6,27,47] Diagnosis is one of exclusion in an infertile bitch and is made on histopathological examination of the ovary.[3,6,28,47] There is no known effective treatment.

References

1. Johnson C: High risk pregnancy and hypoluteoidism in the bitch. Theriogenology 2007;70:1424-1430.
2. Gorlinger S, Galac S, Kooistra HS, et al: Hypoluteoidism in a bitch. Theriogenology 2005;64: 213-219.
3. Johnston SD, Root-Kustritz MV, Olsen PN: Disorders of the canine ovary. Canine and feline theriogenology. Philadelphia: WB Saunders; 2001. p. 193-205.
4. Freshman JL: Clinical approach to infertility in the cycling bitch. Vet Clin N Am Small Anim Prac 1991;21:427-436.
5. Purswell BJ: Management of apparent luteal insufficiency in a bitch. J Am Vet Med Assoc 1991;199:902-903.
6. Grundy SA, Feldman E, Davidson A. Evaluation of infertility in the bitch: Clin Tech Small Anim Pract 2002;17:108-115.
7. Progestins. Drug facts and comparisons. St Louis: Wolters Kluwer Health, April 2008. p 228.
8. Arbeiter K. Anovulatory ovarian cycles in dogs. J Reprod Fertil Suppl 1993;47:453-456.
9. Poffenbarger EM, Feeney DA: Use of gray-scale ultrasonography in the diagnosis of reproductive disease in the bitch: 18 cases (1981-1984). J Am Vet Med Assoc 1986;189: 90-95.
10. Wallace SS, Mahaffey MB, Miller DM, et al: Ultrasonographic appearance of the ovaries of dogs during the follicular and luteal phases of the estrous cycle. Am J Vet Res 1992;53: 209-215.
11. Perkins NR, Thomas PGA: Infertility in the bitch with normal oestrous cycles. Aust Vet Pract 1990;23:77-87.
12. Ball RL, Birchard SJ, May LR, et al: Ovarian remnant syndrome in dogs and cats: 21 cases (2000-2007). J Am Vet Med Assoc 2010;236:548-553.
13. Miller DM: Ovarian remnant syndrome in dogs and cats: 46 cases (1988-1992). J Vet Diagn Invest. 1995;7:572-574.
14. Wallace MS: The ovarian remnant syndrome in the bitch and queen. Vet Clin North Am: Small Anim Prac 1991;21:501-507.
15. Johnston SD, Root-Kustritz MV, Olsen PN: Clinical approach to infertility in the bitch. Canine and feline theriogenology. Philadelphia: WB Saunders; 2001. p. 257-273.
16. Ichimura R, Shibutani M, Mizukami S, et al: A case report of an uncommon sex-cord stromal tumor consisted of luteal and Sertoli cell in a spayed bitch. J Vet Med Sci 2010;72:229-234.
17. Dow C: Ovarian abnormalities in the bitch. J Comp Pathol 1960;70:52-69.
18. McEntee K: Reproductive pathology of domestic mammals. San Diego: Academic Press; 1990. p. 57-93.
19. Nemzek JA, Homco LD, Wheaton LG, et al: Cystic ovaries and hyperestrogenism in a canine female pseudohermphrodite. J Am Anim Hosp Assoc 1992;28:402-406.
20. Fayrer-Hosken RA, Durham DH, Allen S, et al: Follicular cystic ovaries and cystic endometrial hyperplasia in a bitch. J Am Vet Med Assoc 1992;201:107-108.
21. Fiorito DA: Hyperestrogenism in bitches. Compend cont Educ Pract Vet 1992;14:727-729.
22. Farris GA, Benjamin SA: Inhibition of myelopoiesis by serum from dog exposed to estrogen. Am J Vet Res 1993;54:1374-1379.
23. MacLachlan NJ: Ovarian disorders in domestic animals. Environl Health Perspect 1987;73:27-33.
24. Marino G, Mannarino C, Di Prima ML, et al: Stromal cysts in the canine ovary. J Comp Pathol 2009;141:297.
25. Akihara Y, Shimoyama Y, Kawasako K, et al: Immunohistochemical evaluation of canine ovarian cysts. J Vet Med Sci 2007;10:1033-1037.
26. Anderson AC, Simpson ME: Pathology of the ovary and genital tract. The ovary and reproductive cycle of the dog (Beagle). Los Altos: Geron-X; 1973.
27. Johnston SD: Premature gonadal failure in female dogs and cats. J Reprod Fertil Suppl 39;1989;65-72.
28. Johnston SD: Clinical approach to infertility in bitches with primary anestrus. Vet Clin North Am: Small Anim Prac 1991;21:421-426.
29. Lofstedt RM, Buoen LC, Weber AF, et al: Prolonged proestrus in a bitch with X chromosomal monosomy (77, XO). J Am Vet Med Assoc 1992;200:1104-1106.
30. Johnston SD, Buoen LC, Weber AF, et al: X trisomy in an Airedale bitch with ovarian dysplasia and primary anestrus. Theriogenology 1985;24:597-607.
31. Smith FWK, Buoen LC, Weber AF, et al: X-chromosome monosomy (77, XO) in a Doberman Pinscher with gonadal dysgenesis. J Vet Intern Med 1989;3:90-95.
32. Pluhar GE, Memon MA, Wheaton LG: Granulosa cell tumor in an ovariohysterectomized dog. J Am Vet Med Assoc 1995;207:1063-1065.
33. Kennedy PC, Cullen JM, Edwards JF, et al. Histological classification of tumors of the genital system of domestic animals. 2nd ed. Washington DC: Armed Forces Institute of Pathology, American Registry of Pathology, and The World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology; 1998.
34. Patniak AK, Greenlee PG: Canine ovarian neoplasms: a clinicopathologic study of 71 cases, including histology of 12 granulosa cell tumors. Vet Pathol, 1987;24:509-514.
35. Norris HJ, Garner FM, Taylor HB: Comparative pathology of ovarian neoplasms. IV. Gonadal stromal tumors of canine species. J Comp Pathol 1970;80:399-405.
36. Sforna M, Brachelente C, Lepri E,et al: Canine ovarian tumors: a retrospective study of 49 cases. Vet Res Commun. 2003; 27 Suppl 1:359-361.
37. Cotchin E: Canine ovarian neoplasms. Res Vet Sci 1961;2:113-142.
38. McCandlish IA, Munro CD, Breeze RG, et al: Hormone producing ovarian tumors in the dog. Vet Rec 1979;105:9-11.
39. McKee WM: Granulosa cell tumor and attempted chemotherapy in a 17-month-old bitch. Vet Rec 1985;117:501-502.
40. Hayes A, Harvey HJ: Treatment of metastatic granulosa cell tumor in a dog. J Am Vet Med Assoc 1979;174:1304-1306.

41. Allen HL, Franklin GA: Malignant granulosa cell tumor in a bitch. J Am Vet Med Assoc 1975;166:447-448.
42. Greenlee PG, Patniak AK: Canine ovarian tumors of germ cell origin. Vet Pathol 1985;22:117-122.
43. Andrews EJ, Stookey JL, Helland DR, et al: A histopathological study of canine and feline ovarian dysgerminomas. Can J Comp Med 1974;38:85-89.
44. Fernandez T, Diez-Bru N, Rios A, et al. Intracranial metastasis from an ovarian dysgerminoma in a 2-year-old dog. J Am Anim Hosp Assoc 2001;37:553-556.
45. Nagashima Y, Hoshi K, Tanak R, et al: Ovarian and retroperitoneal teratomas in a dog. J Vet Med Sci 2000;62:793-795.
46. Headley SA, Fuck EJ, Fuck ET, et al: Ovarian teratoma in a bitch. Vet Rec 2006;158:565-567.
47. Nickel RF, Okkens AC, van der Gaag I, et al: Oophoritis in a dog with abnormal corpus luteum function. Vet Rec 1991; 128: 333-334.

Optimal age for gonadectomy in dogs and cats

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Introduction

Ovariohysterectomy and castration are the surgeries most commonly performed by small animal practitioners in the United States.[1] Exhaustive reviews of the benefits and detriments of gonadectomy at various ages have been published.[2,3] This is a brief review of the literature to inform decisions regarding best age at which to perform castration or ovariohysterectomy in dogs or cats.

Optimal age at which to perform ovariohysterectomy (OHE) or castration of dogs and cats is not defined by the veterinary literature. In the United States, most veterinarians recommend cats and dogs be spayed or castrated when about six months of age, prior to puberty, which is defined as acquisition of normal breeding behavior and semen quality in males and first estrus in females. In other countries, veterinarians recommend that dogs and cats be spayed after their first estrus, or do not recommend elective surgical sterilization be performed at any age. Indeed, in some countries, elective gonadectomy is considered unethical and is either strongly discouraged or illegal.[4,5] For this discussion, it is assumed that the veterinarian is comfortable with the ethics of elective gonadectomy and practices in a country in which such surgery is considered acceptable by professional associations and society at large.

Dogs and cats can be considered as part of a larger population of animals or as individuals. Recommendation for age at which to perform elective gonadectomy must take this into account. Animals at humane organizations that are not yet associated with a responsible owner or guardian should be evaluated as part of the larger population. Dogs and cats with an owner or guardian may be considered either as part of a larger population or as an individual.

Keywords: Gonadectomy, ovariohysterectomy, castration, neuter, population contol

Dogs and cats with no owner or guardian

In the United States, a serious problem with pet overpopulation exists, such that millions of unowned dogs and cats are euthanized yearly.[6,7] Some of these are feral animals, some are abandoned and brought to the humane association as strays, and many are relinquished. Intact animals are much more likely to be relinquished than are spayed or castrated animals and animals that are adopted out from the humane association while still intact may either be returned or repopulate that shelter with their offspring.[8-10] While most intact animals are adopted out with a spay-neuter contract, compliance with such contracts has been demonstrated to be less than 60%.[11,12] There is a significant lack of knowledge among pet owners regarding normal reproduction; studies have demonstrated that up to 57% of bitch owners were unaware that bitches cycle at least twice yearly, up to 83% of queen owners were unaware that queens are polyestrous from spring to early fall, and up to 61% of dog and cat owners were unsure or believed that their animal would somehow be "better" after having had at least one litter.[8,13,14] In one survey of dog- and cat-owning households, 56% of 154 canine litters and 68% of 317 feline litters were unplanned, with the majority of those owners reporting that they did not know the female had been in heat.[15] While everyone would like to believe that better education of pet owners would lead to more responsible pet ownership, and while increasing education is a worthy goal that should be pursued, gonadectomy of dogs and cats prior to adoption is one weapon in the fight against overpopulation that should be employed at this time. Multiple studies have been published demonstrating safety of gonadectomy in puppies and kittens as young as seven weeks of age.[16-21] To that end, I recommend that all male and female dogs and cats should be spayed or castrated prior to adoption from humane organizations.

Dogs and cats with an owner or guardian

Male cats

The normal behavior of most intact male cats is incompatible with their living as housepets.[22] Breeding behavior in cats is aggressive and intact male cats show that behavior readily. Urine from intact male cats is used for territorial marking and has a very distinct, strong odor. There are many concerns voiced about increased incidence of urinary tract obstruction in castrated male cats due to decrease urethral diameter. Numerous studies have evaluated effect of castration at various ages with urethral diameter and none have documented this correlation.[23-25] There are virtually no health conditions reported to be increased or decreased in association with gonadectomy in male cats. Because of this, I recommend that any male cat not intended for breeding be castrated.

Female cats

Benefits of OHE in female cats include decreased incidence of mammary neoplasia, ovarian or uterine tumors, and pyometra. Of these, the most significant is mammary neoplasia. Mammary neoplasia is the third most common tumor of female cats, with a reported incidence of 2.5%.[26,27] Incidence is increased with number of estrous cycles in the cat's life and is increased in the Siamese and domestic Japanese breeds.[27-29] More than 90% of cases are malignant adenocarcinoma.[26,28,30]

Detriments of OHE in female cats include possible complications of surgery, obesity, increased incidence of feline lower urinary tract disease (FLUTD), and increased incidence of diabetes mellitus. Reported incidence of post-surgical complications in cats is 2.6%, with most reported complications mild and self-resolving.[31] Incidence of obesity after OHE is high, and is due to decreased metabolic rate in cats after gonadectomy.[32,33] Obesity can be controlled by proper feeding regimen. Finally, increased incidence of FLUTD and diabetes mellitus has been reported after OHE in queens, with the Burmese breed especially prone to development of diabetes mellitus.[34-36] Incidence of these two conditions is 0.6% and 0.5%, respectively.[23,34,37]

Because the incidence and morbidity of mammary neoplasia are much higher than are the incidences of FLUTD and diabetes mellitus, and because morbidity associated with obesity can be controlled by the owner or guardian of the cat, I believe that female cats not intended for breeding should be spayed as early in their life as possible.

Male dogs

Benefits of castration in male dogs include decreased incidence of testicular neoplasia and non-neoplastic prostate disease, and possible increased lifespan. Testicular neoplasia is a common tumor of aged, intact male dogs, with a reported incidence of 0.9%.[38] Morbidity generally is low. Benign prostatic hypertrophy (BPH) is a very common disorder of male dogs, with reported incidence of 75–80% in dogs aged six years or more.[39-41] Again, morbidity generally is low. Finally, several studies have documented increased lifespan in castrated male dogs compared to intact males.[42-44] This may be due to greater care by owners after the "investment" of surgery has been made in that animal, or may be due to a decrease in sexually dimorphic behaviors that put the animal at increased risk, such as roaming.

Detriments of castration in male dogs include complications of surgery, increased incidence of prostatic neoplasia,[45-47] transitional cell carcinoma,[48,49] osteosarcoma,[50,51] and hemangiosarcoma,[52,53] increased incidence of anterior cruciate ligament (ACL) injury,[54-56] obesity,[57,58] and possible increased incidence of diabetes mellitus.[59] Reported incidence of post-surgical complications in dogs is 6.1%, with most reported complications mild and self-resolving.[31] Prostatic neoplasia, transitional cell carcinoma, osteosarcoma, and hemangiosarcoma generally are low in incidence but high in morbidity and mortality.[53,60-63] No breed predisposition has been identified for prostatic neoplasia, but does exist for the other cancers noted.[50,64-66] Incidence of ACL injury in dogs is relatively high, at 1.8%, and morbidity may be high, although this is generally considered to be a curable condition with surgery.[54-56] Again, some breeds, most notably large and giant breeds, are predisposed to ACL injury.[56,67,68] Obesity is high in incidence but morbidity can be controlled by the owner or guardian.

Appropriate recommendation for castration of male dogs is less readily evident than is that for male cats. While a given male dog can produce many more offspring than can a given bitch, suggesting that castration is necessary for population control, the significant morbidity associated with castration as a possible predisposing cause of the conditions described above suggests that castration is not recommended when considering the animal as an individual. I believe this recommendation must be made on a case-by-case basis, evaluating the breed of the dog, his intended working life or activity level, ability of the owner to control reproduction in that animal, and the owner's wishes regarding use of that animal for breeding.

Female dogs

Benefits of OHE in bitches include decreased incidence of mammary neoplasia, with greatest benefit if spayed before the first heat, and decreased incidence of ovarian or uterine neoplasia and pyometra.[69] Mammary neoplasia is the most common tumor of female dogs, with reported incidence of 3.4%.[26,70-72] It is the most common malignant tumor in female dogs, with 50.9% of mammary tumors reported to be malignant; metastases are found in about 75% of cases of mammary carcinoma with the lung the most common site of metastasis.[26,73-75] A hormonal basis for malignant transformation of mammary cells and progression of neoplasia is hypothesized based on the decreasing benefit of OHE with increasing number of estrous cycles in the dog's life prior to surgery. The other very common disorder in female dogs when aged is pyometra, reported to occur in 15.2% of dogs by four years of age and in 23–24% of dogs by ten years of age.[76,77] Morbidity is high, although OHE at the time of clinical presentation is curative; reported mortality ranges from 0–17% in dogs.[78]

Detriments of OHE in female dogs include complications of surgery, increased incidence of transitional cell carcinoma,[48,49] osteosarcoma and hemangiosarcoma,[50-53] increased incidence of ACL injury,[54-56] obesity and diabetes mellitus,[57-59] a possible increase in aggression in at least one breed,[79,80] and increased incidence of urethral sphincter mechanism incompetence (estrogen-responsive urinary incontinence).[81-83] Reported incidence of post-surgical complications in dogs is 6.1%, with most reported complications mild and self-resolving.[31] As in male dogs, incidence of tumors reportedly associated with gonadectomy is low but morbidity with these tumor types is high. Breed predispositions exist for all three tumor types. Incidence of obesity is high after OHE but morbidity can be controlled by the owner. Incidence of ACL injury in dogs is relatively high, at 1.8%, and morbidity may be high, although this is generally considered to be a curable condition with surgery. Again, some breeds, most notably large and giant breeds, are predisposed to ACL injury. Aggression after OHE has been reported in English Springer Spaniels; there is some suggestion that this effect may be more likely in bitches that demonstrated aggressive tendencies prior to surgery.[80] Urethral sphincter mechanism incompetence is a problem of spayed female dogs, especially those weighing more than 20 kg.[84] While morbidity is low and this is a disease easily controlled with medical therapy in most female dogs, evidence exists suggesting incidence can be decreased by spaying bitches when greater than three months of age.[85] There is one paper reporting increased lifespan associated with intact status in a population of exceptionally long-lived Rottweilers; significance of these findings to other dog populations is unknown.[86]

Appropriate recommendation for OHE of female dogs is less readily evident than is that for female cats. Certainly mammary neoplasia and pyometra are of high incidence and high morbidity, and are greatly decreased in incidence by OHE. However, possible predisposition to very high morbidity tumor types or ACL injury must be evaluated. As with male dogs, I believe this recommendation must be made on a case-by-case basis, evaluating the breed of the dog, her intended working life or activity level, and the owner's wishes regarding use of that animal for breeding.

Much information and misinformation about this topic is available to the owners, guardians, and breeders of dogs and cats. It behooves us, as veterinarians, to practice evidence-based medicine, the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients.[87] This requires knowledge of the current veterinary literature, including number of studies supporting or refuting an effect of gonadectomy, number and breed of animals in that study, and validity of conclusions drawn. Clients should expect us to base our recommendations on science, rather than on anecdote or tradition.

References

1. Greenfield CL, Johnson AL, Schaeffer DJ: Frequency of use of various procedures, skills, and areas of knowledge among veterinarians in private small animal exclusive or predominant practice and proficiency expected of new veterinary school graduates. J Am Vet Med Assoc 2004;224:1780-1787.
2. Reichler IM: Gonadectomy in cats and dogs: a review of risks and benefits. Reprod Domest Anim. 2009;44:29-35.
3. Root Kustritz MV: Determining the optimal age for gonadectomy of dogs and cats. J Am Vet Med Assoc 2007;231:1665-1675.
4. Gunzel-Apel AR: Early castration of dogs and cats from the point of view of animal welfare. Dtsch Tierarztl Wochenschr 1998;105:95-98.
5. Salmeri KR, Olson PN, Bloomberg MS: Elective gonadectomy in dogs: a review. J Am Vet Med Assoc 1991;198:1183-1192.
6. Nassar R, Talboy J, Moulton C: Animal shelter reporting study 1990, American Humane Association, Englewood CO. 1992:5.
7. National Council on Pet Population Study and Policy. National shelter census: 1994 results, NCCPPSP, Fort Collins CO. 1994:1-2.
8. New JG, Salman MD, Scarlett JM, et al: Shelter relinquishment: characteristics of shelter-relinquished animals and their owners compared with animals and their owners in US pet-owning households. J Appl Anim Welf Sci 2000;3:179-201.
9. Patronek GJ, Glickman LT, Beck AM, et al: Risk factors for relinquishment of dogs to an animal shelter. J Am Vet Med Assoc 1996;209:572-581.
10. Mondelli F, Prato Previde E, Verga M, et al: The bond that never developed: Adoption and relinquishment of dogs in a rescue shelter. J Appl Anim Welf Sci 2004;7:253-266.
11. Alexander SA, Shane SM: Characteristics of animals adopted from an animal control center whose owners complied with a spaying/neutering program. J Am Vet Med Assoc 1994;205:472-476.
12. Eno M, Fekety S: Early age spay/neuter: a growing consensus. Shelter Sense 1993;Nov:1-7.
13. Scarlett JM, Salman MD, New JG, et al: Reasons for relinquishment of companion animals in U.S. animal shelters: selected health and personal issues. J Appl Anim Welf Sci 1999;2:41-57.
14. Scarlett JM, Salman MD, New JG, et al: The role of veterinary practitioners in reducing dog and cat relinquishments and euthanasias. J Am Vet Med Assoc 2002;220:306-311.
15. New JC, Kelch WJ, Hutchison JM, et al: Birth and death rate estimates of cats and dogs in U.S. households and related factors. J Appl Anim Welf Sci 2004;7:229-241.
16. Howe LM: Prepubertal gonadectomy in dogs and cats - part I. Comp Cont Educ Pract Vet 1992;21:103-111.
17. Howe LM: Prepubertal gonadectomy in dogs and cats - part II. Comp Cont Educ Pract Vet 1992;21:197-201.
18. Howe LM Surgical methods of contraception and sterilization. Theriogenology 2006;66:500-509.
19. Howe LM: Short-term results and complications of prepubertal gonadectomy in cats and dogs. J Am Vet Med Assoc 1997;211:57-62.

20. Howe LM, Slater MR, Boothe HW, et al: Long-term outcome of gonadectomy performed at an early age or traditional age in cats. J Am Vet Med Assoc 2000;217:1661-1665.
21. Howe LM, Slater MR, Boothe HW, et al: Long-term outcome of gonadectomy performed at an early age or traditional age in dogs. J Am Vet Med Assoc 2001;218:217-221.
22. Root Kustritz MV: Elective gonadectomy in the cat. Feline Pract 1996;24:36-39.
23. Root MV, Johnston SD, Johnston GR, et al: The effect of prepuberal and postpuberal gonadectomy on penile extrusion and urethral diameter in the domestic cat. Vet Radiol Ultrasound 1996;37:363-366.
24. Spain CV, Scarlett JM, Houpt KA: Long-term risks and benefits of early-age gonadectomy in cats. J Am Vet Med Assoc 2004;224:372-379.
25. Herron MA: The effect of prepubertal castration on the penile urethra of the cat. J Am Vet Med Assoc 1972;160:208-211.
26. Dorn CR, Taylor DO, Frye FL, et al: Survey of animal neoplasms in Alameda and Contra Costa Counties, California. I. Methodology and description of cases. J Natl Cancer Inst 1968;40:295-305.
27. Verstegen J, Onclin K: Mammary tumors in the queen. Proc Annu Meet Soc Therio 2003. p. 239-245.
28. Hayes HM, Milne KL, Mandel CP: Epidemiological features of feline mammary carcinoma. Vet Rec 1981;108:476-479.
29. Dorn CR, Taylor DO, Schneider R, et al: Survey of animal neoplasms in Alameda and Contra Costa Counties, California. II. Cancer morbidity in dogs and cats from Alameda County. J Natl Cancer Inst 1968;40:307-318.
30. Hampe JF, Misdorp W: Tumours and dysplasias of the mammary gland. Bull World Health Organ 1974;50:111-133.
31. Pollari FL, Bonnett BN, Bamsey SC, et al: Postoperative complications of elective surgeries in dogs and cats determined by examining electronic and paper medical records. J Am Vet Med Assoc 1996;208:1882-1886.
32. Root MV, Johnston SD, Olson PN: Effect of prepuberal and postpuberal gonadectomy on heat production measured by indirect calorimetry in male and female domestic cats. Am J Vet Res 1996;57:371-374.
33. Fettman MJ, Stanton CA, Banks LL, et al: Effects of neutering on bodyweight, metabolic rate and glucose tolerance of domestic cats. Res Vet Sci 1997;62:131-136.
34. McCann TM, Simpson KE, Shaw DJ, et al: Feline diabetes mellitus in the UK: the prevalence within an insured cat population and a questionnaire-based putative risk factor analysis. J Feline Med Surg 2007;9:289-299.
35. Prahl A, Guptill L, Glickman NW, et al: Time trends and risk factors for diabetes mellitus in cats presented to veterinary teaching hospitals. J Feline Med Surg 2007;9:351-358.
36. Rand JS, Bobbermien LM, Hendrikz JK, et al: Over representation of Burmese cats with diabetes mellitus. Aust Vet J 1997;75:402-405.
37. Lekcharoensuk C, Osborne CA, Lulich JP: Epidemiologic study of risk factors for lower urinary tract diseases in cats. J Am Vet Med Assoc 2001;218:1429-1435.
38. Hahn KA, von der Haar MA, Teclaw RF: An epidemiological evaluation of 1202 dogs with testicular neoplasia [Abstract]. J Vet Intern Med 1992;6:121.
39. Lowseth LA, Gerlach RF, Gillett NA, et al: Age-related changes in the prostate and testes of the beagle dog. Vet Path 1990;27:347-353.
40. Zirkin BR, Strandberg JD: Quantitative changes in the morphology of the aging canine prostate. Anat Rec 1984;208:207-214.
41. Berry SJ, Strandberg JD, Saunders WJ, et al: Development of canine benign prostatic hyperplasia with age. Prostate 1986;9:363-373.
42. Bronson RT: Variation in age at death of dogs of different sexes and breeds. Am J Vet Res 1982;43:2057-2059.
43. Moore GE, Burkman KD, Carter MN, et al: Causes of death or reasons for euthanasia in military working dogs: 927 cases (1993-1996). J Am Vet Med Assoc 2001;219:209-214.
44. Michell AR: Longevity of British breeds of dog and its relationships with sex, size, cardiovascular variables and disease. Vet Rec 1999;145:625-629.
45. Bryan JN, Keeler MR, Henry CJ, et al: A population study of neutering status as a risk factor for canine prostate cancer. Prostate 2007;67:1174-1181.
46. Sorenmo KU, Goldschmidt M, Shofer F, et al: Immunohistochemical characterization of canine prostatic carcinoma and correlation with castration status and castration time. Vet Comp Oncol 2003;1:48-56.
47. Obradovich J, Walshaw R, Goullaud E: The influence of castration on the development of prostatic carcinoma in the dog: 43 cases (1978-1985). J Vet Intern Med 1987;1:183-187.
48. Norris AM, Laing EJ, Valli VE, et al: Canine bladder and urethral tumors: a retrospective study of 115 cases (1980-1985). J Vet Intern Med 1992;6:145-153.
49. Knapp DW, Glickman NW, DeNicola DB, et al: Naturally-occurring canine transitional cell carcinoma of the urinary bladder. Urol Oncol 2000;5:47-59.
50. Ru G, Terracini B, Blickman LT, et al: Related risk factors for canine osteosarcoma. Vet J 1998;156:31-39.
51. Priester WA, McKay FW: The occurrence of tumors in domestic animals. Natl Cancer Inst Monogr 1980;54:1-210.
52. Prymak C, McKee LJ, Goldschmidt MH, et al: Epidemiologic, clinical, pathologic, and prognostic characteristics of splenic hemangiosarcoma and splenic hematoma in dogs: 217 cases (1985). J Am Vet Med Assoc 1988;193:706-712.
53. Ware WA, Hopper DL: Cardiac tumors in dogs: 1982-1995. J Vet Intern Med 1999;13:95-103.
54. Whitehair JG, Vasseur PB, Willits NH: Epidemiology of cranial cruciate ligament rupture in dogs. J Am Vet Med Assoc 1993;203:1016-1019.
55. Slauterbeck JR, Pankratz K, Xu KT, et al: Canine ovariohysterectomy and orchiectomy increases the prevalence of ACL injury. Clin Orthop Relat Res 2004;429:301-305.
56. Duval JM, Budsberg SC, Flo GL, et al: Breed, sex, and body weight as risk factors for rupture of the cranial cruciate ligament in young dogs. J Am Vet Med Assoc 1999;215:811-814.
57. Crane SW: Occurrence and management of obesity in companion animals. J Sm Anim Pract 1991;32:275-282.
58. Edney AT, Smith PM: Study of obesity in dogs visiting veterinary practices in the United Kingdom. Vet Rec 1986;118:391-396.
59. Marmor M, Willeberg P, Glickman LT, et al: Epizootiologic patterns of diabetes mellitus in dogs. Am J Vet Res 1982;43:465-470.
60. Weaver AD: Fifteen cases of prostatic carcinoma in the dog. Vet Rec 1981;109:71-75.

61. Teske E, Naan EC, van Dijk EM, et al: Canine prostate carcinoma: epidemiological evidence of an increased risk in castrated dogs. Mol Cell Endocrinol 2002;197:251-255.
62. Bell FW, Klausner JS, Hayden DW, et al: Clinical and pathologic features of prostatic adenocarcinoma in sexually intact and castrated dogs: 31 cases (1970-1987). J Am Vet Med Assoc 1991;199:1623-1630.
63. Poirier VJ, Forrest LJ, Adams WM, et al: Piroxicam, mitoxantrone, and coarse fraction radiotherapy for the treatment of transitional cell carcinoma of the bladder in 10 dogs: a pilot study. J Am Anim Hosp Assoc 2004;40:131-136.
64. Smith AN: Hemangiosarcoma in dogs and cats. Vet Clin North Am Small Anim Pract 2003;33:533-52.
65. Henry CJ: Management of transitional cell carcinoma. Vet Clin North Am Small Anim Pract 2003;33:597-613.
66. Chun R, de Lorimier LP: Update on the biology and management of canine osteosarcoma. Vet Clin North Am Small Anim Pract 2003;33:491-516.
67. Harasen G: Canine cranial cruciate ligament rupture in profile: 2002-2007. Can Vet J 2008;49:193-194.
68. Wilke VL, Conzemius MC, Rothschild MF: SNP detection and association analyses of candidate genes for rupture of the cranial cruciate ligament in the dog. Anim Genet 2005;36:519-521.
69. Schneider R, Dorn CR, Taylor DO: Factors influencing canine mammary cancer development and postsurgical survival. J Natl Cancer Inst 1969;43:1249-1261.
70. Richards HG, McNeil PE, Thompson H, et al: An epidemiological analysis of a canine-biopsies database compiled by a diagnostic histopathology service. Prev Vet Med 2001;51:125-136.
71. Moe L: Population-based incidence of mammary tumours in some dog breeds. J Reprod Fertil 2001;Suppl 57:439-443.
72. Fidler IJ, Brodey RS: The biological behavior of canine mammary neoplasms. J Am Vet Med Assoc 1967;151:1311-1318.
73. Brodey RS, Goldschmidt MH, Roszel JR: Canine mammary gland neoplasms. J Am Anim Hosp Assoc 1983;19:61-90.
74. Cotchin E, Douglas SW, Platt H: .Neoplasms in small animals. Vet Rec 1951;63:67-78.
75. Moulton JE, Taylor DO, Dorn CR, et al: Canine mammary tumors. Pathol Vet 1970;7:289-320.
76. Egenvall A, Hagman R, Bonnett BN, et al: Breed risk of pyometra in insured dogs in Sweden. J Vet Intern Med 2001;15:530-538.
77. Fukuda S: Incidence of pyometra in colony-raised beagle dogs. Exp Anim 2001;50:325-329.
78. Johnston SD, Root Kustritz MV, Olson PN: Disorders of the canine uterus and uterine tubes (oviducts). In: Johnston SD, Root Kustritz MV, Olson PN, editors. Canine and feline theriogenology. Philadelphia: WB Saunders; 2001. p. 216.
79. Kim HH, Yeon SC, Houpt KA, et al: Effects of ovariohysterectomy on reactivity in German Shepherd Dogs. Vet J 2006;172:154-159.
80. Reisner IR: Dominance-related aggression of English Springer Spaniels: a review of 53 cases. Appl Anim Behav Sci 1993;37:83-84.
81. Stocklin-Gautschi NM, Hassig M, Reichler IM, et al: The relationship of urinary incontinence to early spaying in bitches. J Reprod Fertil 2001;Suppl 57:233-236.
82. Angioletti A, De Francesco I, Vergottini M, et al: Urinary incontinence after spaying in the bitch: incidence and oestrogen-therapy. Vet Res Commun 2004;28 Suppl 1:153-155.
83. Holt PE: Urinary incontinence in the male and female dog or does sex matter? Available at: www.vin.com. Accessed 10/01, 2004.
84. Holt PE, Thrusfield MV: Association in bitches between breed, size, neutering and docking, and acquired urinary incontinence due to incompetence of the urethral sphincter mechanism. Vet Rec 1993;133:177-180.
85 Spain CV, Scarlett JM, Houpt KA: Long-term risks and benefits of early-age gonadectomy in dogs. J Am Vet Med Assoc 2004;224:380-387.
86. Waters DJ, Kengeri SS, Clever B, et al: Exploring mechanisms of sex differences in longevity: lifetime ovary exposure and exceptional longevity in dogs. Aging Cell 2009;8:752-755.
87. Cockcroft P, Holmes M: Handbook of evidence-based veterinary medicine. Oxford: Blackwell Publishing; 2003.

Pathogenesis of prostatic neoplasia in castrated dogs: why the increased risk?

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Introduction

Prostatic neoplasia in dogs is most often malignant carcinoma. Incidence is increased after castration.[1-4] The disease mimics advanced prostatic neoplasia in men. Parallels will be drawn between the disease in humans and in dogs, possible causes for increased risk after castration in dogs discussed, and potential research avenues described.

Keywords: Prostate, neoplasia, dog

In men, prostatic cancer is the most common neoplasm diagnosed and is the second most common cause of cancer-related death.[5,6] The disease most commonly is diagnosed in men greater than 65 years of age. Autopsy studies have demonstrated that about one-third of men over the age of 50 have some neoplastic change in their prostate and that this increases to about 90% incidence in men aged 90 years or more.[6]

Despite the high incidence of prostatic neoplasia, most men do not die of their disease.[6] Early in the progression of the disease in men, prostatic neoplasia is androgen-dependent.[7] Over time, as androgen-dependent cells die off spontaneously or as a consequence of therapy, a heterogeneous small population of androgen-independent cells undergoes clonal replication.[7] It is unclear whether androgen receptors play into this activation of androgen-independent cells and, if they are involved, if it is due to onset of androgen insensitivity or hypersensitivity.[8,9] This heterogeneous cell population invades locally and metastasizes to distant sites including lymph nodes, lung, bone, and liver.[10] The metastatic cells have a low rate of proliferation and so are relatively resistant to chemotherapy.[11]

Risk factors identified in men for development of prostatic neoplasia include genetics, race, and intact status. Up to 10% of tumors have a hereditary component and incidence is increased in men with an affected father or brother.[12,13] African American men are at greater risk than men of other races.[13,14] Finally, men who never have significant androgen exposure, either because of prepuberal castration or congenital hypogonadism, are at less risk of developing prostatic neoplasia than men who go through puberty and have normal serum concentrations of testosterone and dihydrotestosterone.[14]

In dogs, prostatic neoplasia is an uncommon tumor, with reported incidence ranging from 0.1 to 0.7%.[15,16] The lower incidence in dogs may be due to inability to diagnose the disease until it is advanced enough to cause clinical signs or may be due to different etiology than the disease in men.[17] Diagnosis of prostatic neoplasia is most common in aged dogs, with reported mean age at diagnosis ranging from 8.5 to 9.9 years.[2,16,18] Age at time of diagnosis has not been shown to vary between intact and castrated dogs.[2,4]

Androgen-responsive prostatic neoplasia, as described in men, has not been reported in dogs. The most common cell type and location described are basal cells of ductal or urothelial origin.[1,19] Metastases are most common locally and in lung, liver, spleen, colon and rectum, urethra and urinary bladder, heart, kidney, and bone.[4,20,21] Morbidity and mortality are high; in studies, 76 to 80% of dogs with prostatic carcinoma were euthanized at the time of diagnosis and mean survival of those who lived for more than one week after diagnosis was 30 days with only one dog reported to live for more than four months.[4,22]

Prostatic intraepithelial neoplasia (PIN) is a histologic change in the prostate that some consider to be a pre-neoplastic change in men.[23] In dogs, PIN has been identified that is identical to that in humans.[24] Prostatic intraepithelial neoplasia may be seen in intact or castrated dogs and may or may not be a significant precursor of prostatic neoplasia or a cause of prostatic neoplasia significantly associated with castration in dogs.[24,25]

Age is a risk factor for disease, as described above. Breed has not been demonstrated as a consistent risk factor in dogs; this may be due to difficulty in identifying familial trends with such a low-incidence disorder. A well-recognized risk factor is intact status, usually described as an odds ratio. Odds ratios are calculated by dividing the odds in one group by the odds in another, in this instance by dividing the odds of developing prostatic neoplasia in castrated dogs by the odds of developing prostatic neoplasia in intact dogs. If the odds ratio is greater than one and if the confidence interval for the odds ratio does not include one, a significant risk exists. In dogs, the reported odds ratio for development of prostatic neoplasia after castration varies from 2.1 to 4.3.[1-4] For disorders with low incidence, the odds ratio is virtually identical to the relative risk, suggesting that castrated dogs have a two to four times greater risk of developing prostatic neoplasia than do intact dogs.

Castration is associated with loss of significant secretion of testosterone and its main metabolite, dihydrotestosterone. Lack of androgen feedback to the pituitary is associated with persistent high serum

concentrations of interstitial cell stimulating (luteinizing) hormone.[26] Prostatic atrophy occurs with loss of secretory epithelium and relative increase in the population of proliferative basal cells.[27,28] The epithelium present after castration is less well-differentiated.[29] In dogs castrated after development of prostatic neoplasia, neoplastic cells do not atrophy, further suggesting that it is this highly proliferative basal cell population that is the affected cell type in dogs.[30] Prostatic tumors in castrated dogs are less well-differentiated with a more heterogeneous growth pattern than that seen in affected intact dogs.[4,28,31]

What might be the factors associated with castration that increase neoplastic transformation of prostatic epithelial cells? If decreased circulating androgen is assumed to be causative, one must consider that the great variability between time of castration and onset of tumor suggests castration favors tumor progression, not initiation.[2] One could argue that there may be sources of non-testicular androgen present in circulation but that does not explain the increased incidence in castrated dogs.[18] Androgen deprivation makes cells more resistant to apoptosis, and the loss of androgen and relative increase in serum estrogen may be associated with squamous metaplasia and other pre-neoplastic changes.[3,32,33] The expression of androgen receptors is variable in dogs with prostatic carcinoma, with those receptors capable of binding testosterone, dihydrotestosterone, progesterone, androstane, androstanediol, 17-beta estradiol, and cortisol.[2,34] The androgen receptor pathway also is associated with expression of polypeptide regulatory proteins, membrane-bound receptors, and metabolic enzymes that may be altered, with subsequent changes in other processes along the androgen signaling pathway.[9]

If androgen deprivation is not the link between castration and neoplastic change in the prostate, could it be simply that castration is associated with a larger population of rapidly dividing basal cells? Other factors also may be implicated; castration is associated with an increased number of endothelin receptors on the prostate, which is associated with uncontrolled cell growth and increased osteoblast function, promoting metastasis.[35,36]

One could turn the question around and ask why is prostatic neoplasia less common in dogs left intact? Possibilities include a protective effect of androgens by maintenance of a slowly growing secretory epithelial cell population, or perhaps to the negative association of intact status with longevity.[37-40]

Can we use humans as a model of animal disease and extrapolate information about prevention and treatment from the human literature? A comparison of human and canine prostatic neoplasia suggests not (Table 1).[37] Future research should be focused on better identifying the apparent link between the decline in androgen secretion and increased incidence of prostatic neoplasia. Possible studies include characterization of circulating and intraprostatic concentrations of steroid hormones, their binding to prostatic steroid receptors, and identification of processes signaled by that binding, and treatment response to androgens in dogs with prostatic neoplasia. Measurement of luteinizing hormone receptors on the basal cell population and effect, if any, of their stimulation should be investigated. Use of gonadotropin releasing hormone analogs to downregulate luteinizing hormone secretion as a therapy may be useful. The author hopes to undertake a study better characterizing metabolic change within the prostate as a marker of pre-neoplastic change in castrated and intact dogs. Another illuminating study would be prostatic biopsy of castrated and intact dogs with and without prostatic neoplasia, again to try to better identify pre-neoplastic change. If pre-neoplastic disease or early onset disease could be identified, associated risk factors may be more evident and disease prevented.

Table 1. Comparison of prostatic neoplasia in humans and dogs

	HUMAN	CANINE
AGE AT DISEASE ONSET	Present in about 1/3 of men aged 50 years or more	Greater than 9 years (equivalent of 50 to 52 human years)
HEREDITARY PREDISPOSITION	Genetics, race	None reported
EFFECT OF ANDROGEN DEPRIVATION	Incidence decreased with castration, congenital hypogonadism	Incidence increased with castration
ANDROGEN INFLUENCE	Significant early, not significant late in disease course	Not significant at any point
DISEASE PROGRESSION	Neoplasia aggressive and metastatic only late in disease course	Neoplasia aggressive and metastatic from diagnosis

References

1. Sorenmo KU, Goldschmidt M, Shofer F, et al: Immunohistochemical characterization of canine prostatic carcinoma and correlation with castration status and castration time. Vet Comp Oncol 2003;1:48-56.
2. Teske E, Naan EC, van Dijk EM, et al: Canine prostatic carcinoma: Epidemiological evidence of an increased risk in castrated dogs. Mol Cell Endocrinol 2002;197:251-255.
3. Bryan JN, Keeler MR, Henry CJ, et al: A population study of neutering status as a risk factor for canine prostatic cancer. Prostate 2007;167:1174-1181.

4. Bell FW, Klausner JS, Hayden DW, et al: Clinical and pathologic features of prostatic adenocarcinoma in sexually intact and castrated dogs: 31 cases (1970-1987). J Am Vet Med Assoc 1991;199:1623-1630.
5. Carter BS, Carter HB, Isaacs JT: Epidemiologic evidence regarding predisposing factors to prostatic cancer. Prostate 1990;16:187-197.
6. Garnick MB: The dilemmas of prostate cancer. Sci Am 1994;270:72-81.
7. Ward WS: Building the road to defeat prostate cancer: In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 3-10.
8. Visakorpi T: AR gene alterations in prostate cancer progression. In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 29-37.
9. Febbo PG, Kantoff PW: Androgen receptor polymorphisms and prostate cancer risk. In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 95-109.
10. Bova GS, Chan-Tack KM, Lecates WW: Lethal metastatic human prostate cancer. In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 39-60.
11. Joseph IBJK, Isaacs JT: Angiogenesis and prostate cancer. In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 279-306.
12. Isaacs WB, Xu J, Walsh PC: Hereditary prostate cancer. In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 13-28.
13. Carter BS, Beaty TH, Stewberg GD, et al: Mendelian inheritance of familial prostate cancer. Proc Natl Acad Sci USA 1992;89:3367-3371.
14. Ross RK, Reichardt JKV, Ingles SA, et al: The genetic epidemiology of prostate cancer. In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 111-121.
15. Merlo DF, Rossi L, Pellegrino C, et al: Cancer incidence in pet dogs: findings of the animal tumor registry of Genoa, Italy. J Vet Intern Med 2008;22:976-984.
16. Hargis AM, Miller LM: Prostatic carcinoma in dogs. Compend Cont Educ Pract Vet 1983;5:647-653.
17. Leav I, Ling GV: Adenocarcinoma of the canine prostate. Cancer 1968;22:1329-1345.
18. Obradovich J, Walshaw R, Goullaud E: The influence of castration on the development of prostatic carcinoma in the dog: 43 cases (1978-1985). J Vet Intern Med 1987;1:183-187.
19. Leav I, Schelling KH, Adams JY, et al: Role of canine basal cells in prostatic post natal development, induction of hyperplasia, sex hormone-stimulated growth, and the ductal origin of carcinoma. Prostate 2001;47:149-163.
20. Durham SK: Prostatic adenocarcinoma with and without metastasis to bone in dogs. J Am Vet Med Assoc 1986;188:1432-1436.
21. Taylor PA: Prostatic adenocarcinoma in a dog and a summary of ten cases. Can Vet J 1973;14:162-166.
22. Mann FA, Barrett RJ, Henderson RA: Use of a retained urethral catheter in three dogs with prostatic neoplasia. Vet Surg 1992;21:342-347.
23. Duray PH, Ornstein DK, Vocke CD, et al: Genetic alterations in prostatic intraepithelial neoplasia (PIN). In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 141-161.
24. Waters DJ, Bostwick DG: Prostatic intraepithelial neoplasia occurs spontaneously in the canine prostate. J Urol 1997;157:713-716.
25. Waters DJ, Hayden DW, Bell FW, et al: Prostatic intraepithelial neoplasia in dogs with spontaneous prostate cancer. Prostate 1997;30:92-97.
26. Gunzel-Apel A-R, Seefeldt A, Eschricht FM, et al: Effects of gonadectomy on prolactin and LH secretion and the pituitary-thyroid axis in male dogs. Theriogenology 2008;71:746-753.
27. Mahapokai W, Xue Y, van Garderen E, et al. Cell kinetics and differentiation after hormonal-induced prostatic hyperplasia in the dog. Prostate 2000;44:40-48.
28. Lai CL, van den Ham R, van Leenders G, et al: Histopathological and immunohistochemical characterization of canine prostate cancer. Prostate 2008;68:477-488.
29. Lai CL, van den Ham R, van Leenders G, et al: Comparative characterization of the canine normal prostate in intact and castrated animals. Prostate 2008;68:498-507.
30. Leav I, Cavazos LF, Ofner P: Fine structure and C_{19}-steroid metabolism of spontaneous adenocarcinoma of the canine prostate. J Natl Cancer Inst 1974;52:789-797.
31. Cornell KK, Bostwick DG, Cooley DM, et al: Clinical and pathological aspects of spontaneous canine prostate carcinoma: a retrospective analysis of 76 cases. Prostate 2000;45:173-183.
32. Hsieh J-T: The role of cell adhesion molecules in prostate development and carcinogenesis. In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 207-218.
33. Dore M, Chevalier S, Sirois J: Estrogen-dependent induction of cyclooxygenase-2 in the canine prostate in vivo. Vet Pathol 2005;42:100-103.
34. Mora GR, Tindall DJ: Ligand dependent and independent activation of the androgen receptor. In: Chung LWK, Isaacs WB, Simons JW, editors. Prostate cancer: biology, genetics, and the new therapeutics. Totowa (NJ): Humana Press; 2001. p. 219-239.
35. Padley RJ, Dixon DB, Wu-Wong JR: Effect of castration on endothelin receptors. Clin Sci 2002;103 (Suppl 48):442S-445S.
36. LeRoy BE, Sellers RS, Rosol TJ: Canine prostate stimulates osteoblast function using the endothelin receptor. Prostate 2004;59:148-156.
37. LeRoy BE, Northrup N: Prostate cancer in dogs: comparative and clinical aspects. Vet J 2009;180:149-162.
38. Moore GE, Burkman KD, Carter MN, et al: Causes of death or reasons for euthanasia in military working dogs: 927 cases (1993-1996). J Am Vet Med Assoc 2001;219:209-214.
39. Bronson RT: Variation in age at death of dogs of different sexes and breeds. Am J Vet Res 1982;43:2057-2059.
40. Michell AR: Longevity of British breeds of dog and its relationship with sex, size, cardiovascular variables and disease. Vet Rec 1999;145:625-629.

Control of prolactin secretion in canine hypothyroidism

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Abstract

Hypothyroidism in bitches has been reported to cause elevated serum prolactin concentration and reproductive abnormalities. Dopamine and thyrotropin releasing hormone (TRH) are important factors in normal prolactin homeostasis. The objective of this study was to evaluate the effect of experimentally induced hypothyroidism in bitches on prolactin stimulation and suppression. Eighteen healthy multiparous bitches were used; hypothyroidism was induced (by radioiodine administration) in nine bitches and the remaining nine served as untreated controls. During anestrus, each bitch was treated with TRH (10 μg/kg intravenously), metoclopramide (0.4 mg/kg intravenously), and cabergoline (5 μg/kg subcutaneously) at one week intervals between treatments. Serum prolactin was measured using a commercially available assay. Basal prolactin concentrations were not affected by hypothyroidism. Prolactin was increased in hypothyroid as compared to control bitches after administration of metoclopramide and TRH; and a greater effect was seen after metoclopramide administration as compared to TRH. Suppression of prolactin by cabergoline administration was greater in control than in hypothyroid bitches. Prolactin secretion is increased in hypothyroid bitches during anestrus and may contribute to reproductive failure seen in hypothyroidism.

Keywords: Hypothyroidism, bitch, prolactin, dopamine, TRH

Introduction

Hypothyroidism is reported to be a common cause of reproductive abnormalities in dogs. However, the effects of hypothyroidism on reproduction in the bitch have not been well documented.[1,2] Reported findings include infertility, prolonged anestrus, early embryonic death or abortion, and stillbirth.[2,3] Recently, short-term hypothyroidism in bitches has been found to result in lower birth weight and increased periparturient mortality, while hypothyroidism of approximately one year duration resulted in infertility.[4,5]

Prolactin is the primary luteotrophic factor in the bitch,[6] and inhibition of prolactin secretion shortens interestrus intervals.[7] Prolactin is also responsible for lobuloalveolar development of mammary tissue.[8] Hyperprolactinemia is reported to occur hypothyroidism, and may be associated with inappropriate galactorrhea.[9-12] The role of prolactin in reproductive failure associated with hypothyroidism is unclear, but hyperprolactinemia may be associated with ovulatory dysfunction and infertility.

Prolactin secretion is controlled primarily by dopaminergic inhibition, and also by TRH and other releasing factors.[13] Elevated TRH in hypothyroidism may be the cause of hypothyroid-associated hyperprolactinemia, and subsequent reproductive failure. In this study, we investigated the effect of short-term hypothyroidism during anestrous in bitches on regulation of prolactin secretion by TRH and dopamine.

Materials and methods

Animals

Eighteen intact adult female mongrels, 2-3.5 years old, 8-13 kg body weight, were obtained from a commercial breeder (Covance, Cumberland, VA, USA). Dogs were housed in indoor runs at 21 °C, with a 12 hour light:dark cycle. All bitches were determined to be clinically normal, based on lack of significant abnormalities on physical examination, complete blood count, serum biochemistry, urinalysis, heartworm antigen test, and zinc sulfate fecal floatation. Serum concentrations of total thyroxine (T4), free T4 by equilibrium dialysis, and endogenous canine thyroid stimulating hormone (TSH) were within respective reference ranges. All bitches had previously produced at least two normal litters. Dogs were fed a commercial maintenance diet (Hill's Science Diet Adult dry kibble, Topeka, KS) and offered water ad libitum. The study was approved by the Virginia Tech Animal Care and Use Committee.

Induction of hypothyroidism

Following 12-18 weeks of acclimation and data collection, hypothyroidism was induced in nine randomly selected bitches by intravenous administration of 1mCi/kg ^{131}Iodine (Cardinal Health, Charlottesville, VA). Hypothyroidism was confirmed 9 weeks and 38-45 weeks after ^{131}I by measurement of serum T4 concentrations <10 nmol/L before and 4 hours after administration of 50 ug human recombinant TSH (Thyrogen®, Genzyme Corp., Framingham, MA). The remaining nine untreated bitches acted as controls.

Experimental protocol

Dogs were determined to be in anestrous based on at least 90 days beyond onset date of the most recent estrus, noncornified vaginal cytology, and serum progesterone less than 1 ng/ml (Target®, Biometalics, Inc., Princeton, NJ). Testing began at a mean of 39 weeks (range 33-45) from induction of hypothyroidism. Each dog was subjected to testing with one of three substances (thyrotropin releasing hormone, metoclopramide, and cabergoline) in a randomized design with a one-week interval between treatments. Sampling occurred at 0, 10, 20, 30, 45, 60, 90 minutes after intravenous administration of TRH (10 ug/kg; Sigma Chemical, St. Louis, MO) and metoclopramide (0.4 mg/kg; Reglan®, Baxter Healthcare, Deer Park, IL), and at 0, 4, 8, 12, 24, 36, 48, 60 hours after subcutaneous administration of cabergoline (5 ug/kg; Galastop®, CEVA VETEM, Milano, Italy). Time 0 samples were obtained immediately prior to treatment. Blood samples (8 ml) were collected by jugular venipuncture and allowed to clot at room temperature for 30 minutes prior to centrifugation at 1200 x g for 20 minutes. Serum was then divided into aliquots and stored at -70 ºC until assayed.

Sample analysis

Serum prolactin concentrations were measured using a previously validated[14] commercial homologous canine prolactin enzyme immunoassay (ALPCO Diagnostics, Windham, NH), as described by the manufacturer. Interassay coefficient of variation was 12% and intraassay coefficient of variation was 9%. Prolactin assays were performed at the Small Animal Reproduction Clinic Endocrinology Laboratory, University of Florida College of Veterinary Medicine.

Statistical analysis

Area under the curve (AUC) of prolactin concentration during each test was calculated using the trapezoidal rule. Results of baseline concentrations were excluded from calculation of AUC in the TRH and metoclopramide response tests. Values of prolactin >160 ng/ml were assigned a value of 160 ng/ml and those <0.3 ng/ml were assigned a value of 0.3 ng/ml for purposes of analysis. Testing for Gaussian distribution was accomplished using the Shapiro-Wilk test. Comparisons between groups were accomplished by ANOVA using a general linear model (SAS Enterprise, SAS Institute Inc., Cary, NC). The Wilcoxon sign-rank test was used for data that was not normally distributed. Serum prolactin concentrations were compared on basal samples, and the difference between basal and subsequent prolactin concentrations after administration of test substances. Comparisons of absolute and proportional change in prolactin from time 0 in response to stimulation and suppression were made between the control and hypothyroid groups. The proportional change between hypothyroid and control groups for AUC of prolactin in response to TRH and metoclopramide was compared using ANOVA. The prolactin response to TRH and metoclopramide were also compared. Level of significance was set at $P<0.05$. Values are expressed as mean +/- SD unless otherwise stated.

Results

At the time of testing, all hypothyroid dogs showed clinical signs of hypothyroidism including weight gain, thin hair coat or alopecia, and lethargy. Galactorrhea was not noted in any dog. Serum T4 concentrations before and after TSH administration were <5 nmol/L in all hypothyroid dogs at both 9 and 38-45 weeks after ^{131}I administration. In control dogs, all post-TSH serum T4 concentrations were >35 nmol/L. The mean +/- SD serum T4 concentrations before and after TSH in control dogs was 25 +/- 11 nmol/L and 59 +/- 14 nmol/L at 9 weeks, and 25 +/- 7 and 59 +/- 17 at 38-45 weeks, respectively. Emesis occurred in 9 of 18 dogs within 4 hours following cabergoline administration. No significant side effects were seen with administration of TRH or metoclopramide.

With the exception of differences between AUC of TRH and metoclopramide tests, data were found to be normally distributed. Prolactin concentration in response to TRH was log-transformed prior to analysis. There was no difference in baseline prolactin concentration between the hypothyroid and control groups. The mean serum prolactin concentration increased above baseline after TRH and metoclopramide administration in both hypothyroid ($P=0.0001$, $P<0.0001$, respectively) and control ($P=0.02$, $P=0.02$, respectively) groups (Fig. 1). The mean serum prolactin concentration 90 minutes after TRH administration was higher in hypothyroid than control dogs ($P=0.02$).

Following metoclopramide administration, the mean serum prolactin concentration was higher in hypothyroid than control dogs at all sample times ($P<0.001$), and remained significantly above the baseline concentration throughout the 90 minute sampling period (Fig. 2). Serum prolactin concentrations were decreased from basal concentrations in both control and hypothyroid groups at 4 to 36 (both $P<0.05$) hours after cabergoline administration (Fig. 3). The proportional change in prolactin concentrations from baseline was greater in control as compared to hypothyroid dogs at 24 ($P=0.029$), 36 ($P=0.0087$), and 60 ($P=0.021$) hours after cabergoline administration. No significant difference was found between hypothyroid and control dogs in AUC analysis of prolactin concentrations after TRH administration ($P=0.09$, Table). AUC was different between hypothyroid and control dogs after administration of metoclopramide ($P<0.0001$) and cabergoline ($P<0.0001$). When the relative difference of the AUC of prolactin in hypothyroid and control dogs was compared for metoclopramide (6.7+/- 2.6 times) and TRH (2.1 +/- 2.0 times), the difference was significant ($P<0.005$).

Discussion

Results of the present study show that prolactin secretion is increased in female hypothyroid dogs during anestrus. The increased prolactin secretion in response to TRH administration is similar to that previously found in dogs with experimental and spontaneous hypothyroidism.[9,10,15] Hypothyroidism is associated with decreased negative feedback of thyroid hormones to the hypothalamus, resulting in an increase in TRH secretion into the hypothalamo-hypophyseal portal system. Prolactin secretion is increased at least in part as a result of the stimulatory effect of TRH on pituitary lactotrophs. Diaz-Espineira, et al. reported that the increase in prolactin secretion in response to TRH peaked six months after induction of hypothyroidism before declining thereafter. Because the present study evaluated prolactin response to TRH at a single time and used intact bitches, while dogs in the longer duration study were ovariectomized, it is not possible to make direct comparisons of results.

Basal prolactin concentration was not affected by hypothyroidism in the present study, while previous studies in dogs have reported increased, unchanged, or decreased basal prolactin. The differences between the studies may be due to the influence of the duration of hypothyroidism, gender, and stage of estrus.[9,10,15] In a previous study of experimental hypothyroidism, basal prolactin was unchanged for the first six months of hypothyroidism, then decreased significantly thereafter for the next 2.5 years.[9] Because the present study was carried out nine months after induction of hypothyroidism and comprised one time point, a similar trend cannot be determined. Patients with spontaneous hypothyroidism will likely not be examined until they are exhibiting classic signs of disease, as long as one to two years after actual onset of clinical signs of hypothyroidism in one study.[15] Therefore, the effect of more prolonged hypothyroidism on prolactin secretion may differ from the results reported here. Serum prolactin concentration varies depending on the stage of estrus in normal bitches and has been suggested to affect prolactin levels and responsiveness in previous studies of hypothyroid dogs.[15,16] The present study attempted to control for this effect by testing bitches only during anestrus, and further investigation will be necessary to determine the effects of hypothyroidism on prolactin secretion in other stages of estrus.

In addition to basal prolactin and response to TRH, the present study evaluated the influence of dopamine as a regulator of prolactin secretion. Prolactin secretion in mammals is primarily controlled by the inhibitory effects of dopamine, through tuberoinfundibular dopamine (TIDA) neurons, on the otherwise unrestrained secretion by lactotrophs in the anterior pituitary gland. Using metoclopramide and cabergoline as dopamine-2 receptor antagonist and agonist, respectively, prolactin secretion was altered. Control of prolactin secretion in hypothyroid dogs appears particularly sensitive to dopaminergic control. Hypothyroidism induced an increase in prolactin secretion in response to metoclopramide that was 3.2 times greater than that caused by TRH when results were normalized by that of control dogs. This may indicate increased sensitivity to dopamine or merely hyperplasia of lactotrophs resulting in increased secretory capacity that is revealed when the restraint of dopamine on secretion is removed. The role of altered TIDA neuron activity as a cause of increased prolactin secretion in hypothyroidism is unclear, but it was recently shown to not be altered in hypothyroid rats.[17]

In addition to the control of prolactin secretion by TRH and dopamine, other factors have been demonstrated to stimulate its release. Vasoactive intestinal peptide (VIP) increases prolactin secretion by lactotrophs at concentrations present in the anterior pituitary.[18] Hypothyroidism causes an increase in VIP in the anterior pituitary gland of hypothyroid rats with increased prolactin secretion in the absence of altered TIDA activity.[17,19] The significance of other prolactin-releasing substances, including serotonin, vasoactive intestinal peptide, endothelin, prolactin releasing peptide, oxytocin, and endogenous opiods, is unknown in the pathogenesis of increased prolactin secretion in hypothyroidism.

Pulsatile release results in great variability in basal serum prolactin levels. Levels may also vary within a group of dogs and between laboratories.[20] Handling stress is also shown to transiently affect prolactin release in some studies. This study accounted for these influences on serum prolactin by using AUC and proportional change

for comparison as well as comparisons with a control group treated in an identical manner. In order to reduce stress, all dogs in this study had been extensively acclimated to handling and sampling procedures. Similar testing with saline administration (unpublished data) showed no significant change from baseline due to pulsatile release or handling stress in this group of dogs.

Galactorrhea was not seen in any bitches in this study. The inconsistent signs related to hyperprolactinemia in hypothyroid bitches and the occurrence of hyperprolactinemia in only a portion of untreated hypothyroid women is evidence for a dominant effect of dopamine over TRH for control of prolactin secretion.[11,12,21] Failure to identify elevated basal prolactin concentrations in hypothyroid dogs in the present study may account for the lack of galactorrhea. Based on current understanding of the functions of prolactin, enhanced prolactin secretion in hypothyroid bitches has the potential to cause inappropriate galactorrhea, prolonged anestrus, and early embryonic death, abortion, or stillbirth. While early studies (19 weeks after induction of hypothyroidism) in the dogs used in the present study documented only lower birth weight, and increased periparturient mortality, more prolonged hypothyroidism (one year duration) resulted in infertility in addition to the abnormalities noted earlier.[4,5]

In conclusion, basal prolactin concentrations were unchanged in hypothyroid dogs, while the response to TRH and sensitivity to dopaminergic control were increased. The role that altered prolactin secretion has on reproduction is unknown, but may include infertility and decreased puppy viability. Further study is necessary to fully characterize the effects of hypothyroidism on prolactin regulation and to understand the possible clinical implications.

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References

1. Johnson CA: Thyroid issues in reproduction. Clin Tech Small Anim Pract 2002;17:129-132.
2. Johnson CA: Reproductive manifestations of thyroid disease. Vet Clin North Am Small Anim Pract 1994;24:509-514.
3. Fontbonne A, Siliart B, Badinand F: Hormonal findings in dogs and bitches showing reproductive disorders. J Reprod Fertil Suppl 1993;47:553-554.
4. Panciera DL, Purswell BJ, Kolster KA: Effect of short-term hypothyroidism on reproduction in the bitch. Theriogenology 2007;68:316-321.
5. Panciera DL, Purswell BJ, Kolster KA: Effect of hypothyroidism on reproduction in bitches. J Vet Intern Med 2008;22:726.
6. Okkens AC, Bevers MM, Dieleman SJ, et al: Evidence for prolactin as the main luteotrophic factor in the cyclic dog. Vet Q 1990;12:193-201.
7. Verstegen JP, Onclin K, Silva LD, et al: Effect of stage of anestrus on the induction of estrus by the dopamine agonist cabergoline in dogs. Theriogenology 1999;51:597-611.
8. Kooistra HS, Okkens AC: Secretion of growth hormone and prolactin during progression of the luteal phase in healthy dogs: a review. Mol Cell Endocrinol 2002;197:167-72.
9. Diaz-Espineria MM, Mol JA, van den Ingh TS, et al: Functional and morphological changes in the adenohypophysis of dogs with induced primary hypothyroidism: loss of TSH hypersecretion, hypersomatotropism, hypoprolactinemia, and pituitary enlargement with transdifferentiation. Domest Anim Endocrinol 2008;35:98-111.
10. Kaufman J, Olson PN, Reimers TJ, et al: Serum concentrations of thyroxine, 3,5,3'-triiodothyronine, thyrotropin, and prolactin in dogs before and after thyrotropin-releasing hormone administration. Am J Vet Res 1985;46:486-492.
11. Chastain CB, Schmimdt B: Galactorrhea associated with hypothyroidism in intact bitches. J Am Anim Hosp Assoc 1980;16:851-854.
12. Cortese L, Oliva G, Verstegen J, et al: Hyperprolactinemia and galactorrhoea associated with primary hypothyroidism in a bitch. J Small Anim Pract 1997;38:572-575.
13. Fitzgerald P, Dinan TG: Prolactin and dopamine: what is the connection? A review article. J Psychopharmacol 2008;22:12-19.
14. Onclin K, Verstegen JP: In vivo investigation of luteal function in dogs: effects of cabergoline, a dopamine agonist, and prolactin on progesterone secretion during mid-pregnancy and -diestrus. Domest Anim Endocrinol 1997; 14:25-38.
15. Diaz-Espineira MM, Mol JA, Rijnberk A, et al: Adenohypophyseal function in dogs with primary hypothyroidism and nonthyroidal illness. J Vet Intern Med 2009;23:100-107.
16. Kooistra HS, Okkens AC: Secretion of prolactin and growth hormone in relation to ovarian activity in the dog. Reprod Domest Anim 2001;36:115-119.
17. Tohei A, Taya K, Watanabe G, et al: Hypothyroidism increases prolactin secretion and decreases the intromission threshold for induction of pseudopregnancy in adult female rats. Physiol Behav 2000;69:391-397.
18. Samson WK, Said SI, Snyder G, et al: In vitro stimulation of prolactin release by vasoactive intestinal peptide. Peptides 1980;1:325-332.
19. Lam KS, Lechan RM, Minamitani N, et al: Vasoactive intestinal peptide in the anterior pituitary is increased in hypothyroidism. Endocrinology 1989;124:1077-84.
20. Gobello C, Bolognani F, de la Sota RL, et al: Twenty-four-hour profiles of serum prolactin and luteinizing hormone in anoestrous crossbred bitches. Reprod Domest Anim 2001;36:41-5.
21. Honbo KS, van Herle AJ, Kellett KA: Serum prolactin levels in untreated primary hypothyroidism. Am J Med 1978;64:782-7.

Figure 1. Serum prolactin response to TRH.
Mean +/- SD serum prolactin concentration in 9 euthyroid control (dashed line) and 9 hypothyroid (solid line) bitches in response to intravenous administration of TRH (10 µg/kg). Asterisk indicates a significant difference (P=0.02) between groups.

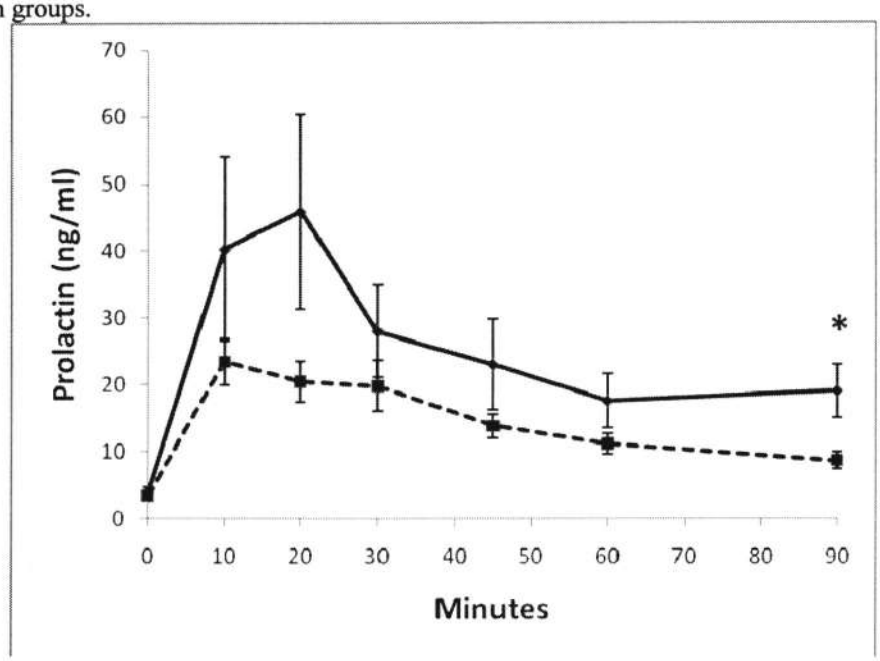

Figure 2. Serum prolactin response to metoclopramide.
Mean +/- SD serum prolactin concentration in 9 euthyroid control (dashed line) and 9 hypothyroid (solid line) bitches in response to intravenous administration of metoclopramide (0.4mg/kg). Asterisks indicate a significant difference (P<0.0001) between groups.

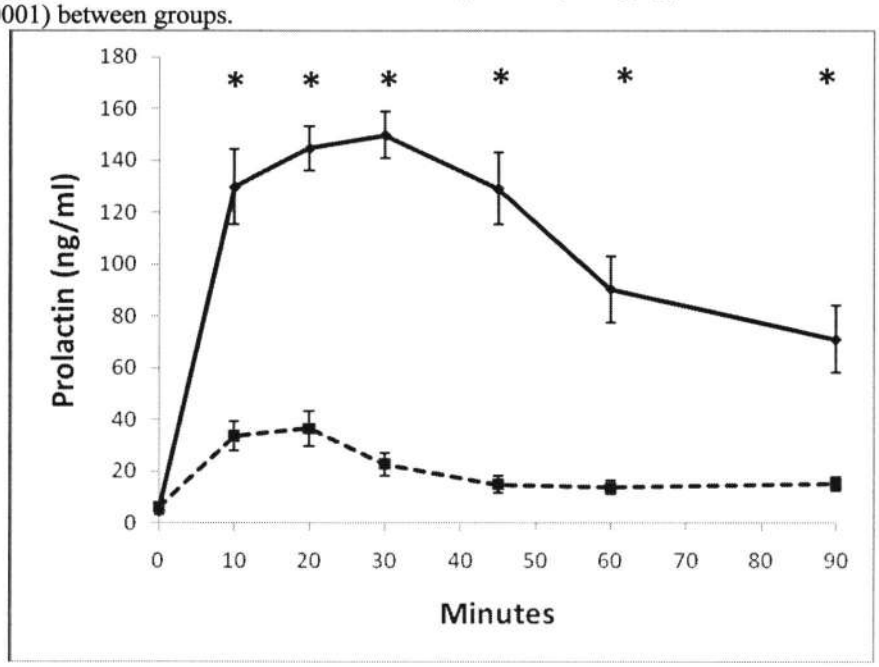

Figure 3. Serum prolactin response to cabergoline.
Mean +/- SD serum prolactin concentration in 9 euthyroid control (dashed line) and 9 hypothyroid (solid line) bitches in response to subcutaneous administration of cabergoline (5 μg/kg). Asterisks indicate a significant difference (P<0.05) between groups.

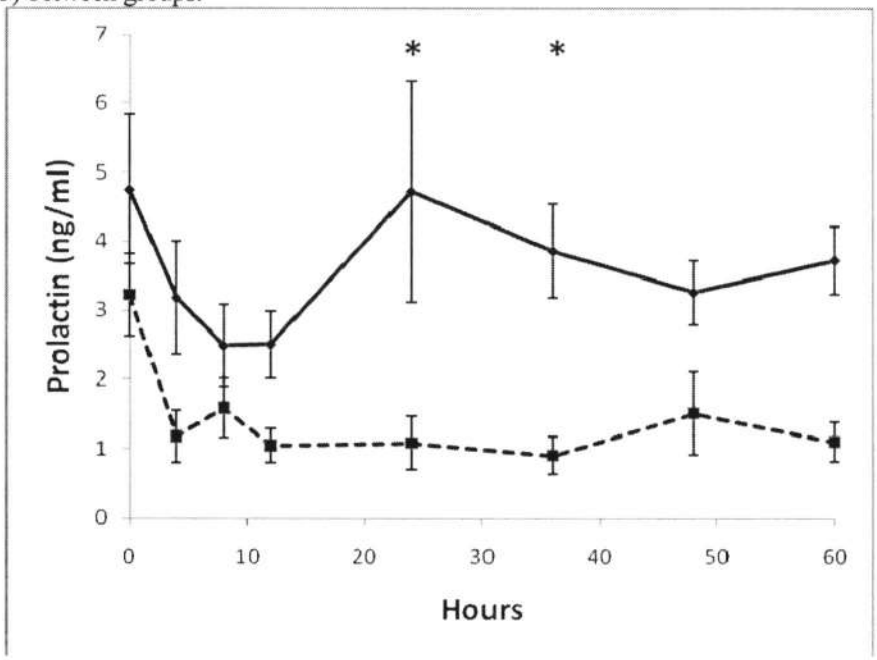

Table 1. Area under the curve (AUC) results for prolactin response tests.
Mean +/- SD prolactin concentration (ng/ml). Results within a row with different superscripts are significantly different (P<0.05).

	Hypothyroid	Control
TRH	2259 ± 1782^{a}	1293 ± 517^{a}
Metoclopramide	9343 ± 2741^{a}	1783 ± 923^{b}
Cabergoline	210 ± 83^{a}	74 ± 52^{b}

Effect of estrus induction on pregnancy rates in domestic bitches and queens

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Abstract

Reported methods for estrus induction in bitches and queens include the use of synthetic estrogens (diethylstilbesterol), dopamine agonists (bromocriptine and cabergoline), gonadotropin releasing hormone agonists (lutrelin, buserelin, fertirelin, deslorelin, and leuprolide), exogenous gonadotropins (luteinizing hormone, follicle stimulating hormone, human chorionic gonadotropin, pregnant mare serum gonadotropin, and human menopausal gonadotropin) and opiate antagonists (naloxone). These methods vary widely in efficacy of inducing estrus as well as in the pregnancy rates following the induced estrus. The applicability of some of these methods for clinical practice is questionable. This review will summarize published reports on estrus induction protocols in domestic bitches and queens for which pregnancy rates are known. Only a brief overview of normal female canine and feline estrous cycle physiology will be discussed and non-medical methods of estrus induction (e.g. dormitory effect in the bitch and light therapy in the queen) will be excluded.

Keywords: Cat, dog, estrus induction, fertility, pregnancy rate

Introduction

Indications for estrus induction in the dog and cat include potential missed breeding opportunities or conception failure and the treatment of primary or secondary anestrus. Estrus induction improves the management of breeding colonies (number of litters per year), especially in establishments where a continuous supply of pups is required (e.g. service dog industry). In the research laboratory, it is often desirable to control the timing of pregnancy so that parturition occurs at the same time. In addition, reliable synchronous estrus induction is a necessity for synchronization of ovulation for embryo transfer programs. Estrus induction is also an effective tool for teaching veterinary reproduction.

Reported methods for estrus induction in bitches and queens include the use of synthetic estrogens (diethylstilbesterol), dopamine agonists (bromocriptine and cabergoline), gonadotropin-releasing hormone (GnRH) agonists (lutrelin, buserelin, fertirelin, deslorelin, and leuprolide), exogenous gonadotropins (luteinizing hormone [LH], follicle-stimulating hormone [FSH], human chorionic gonadotropin [hCG], pregnant mare serum gonadotropin [PMSG], and human menopausal gonadotropin [hMG]) and opiate antagonists (naloxone). These methods vary widely in efficacy of inducing estrus as well as in the pregnancy rates following the induced estrus. The applicability of some of these methods for clinical practice is questionable.

This review will summarize published reports on estrus induction protocols in domestic bitches and queens for which pregnancy rates are known. Only a brief overview of normal female canine and feline estrous cycle physiology will be discussed and non-medical methods of estrus induction (e.g. dormitory effect in the bitch and light therapy in the queen) will be left out.

Canine reproductive physiology

Canine reproductive physiology has unique characteristics that make extrapolation from farm animals unsuccessful in this species. Domestic bitches are nonseasonally monoestrous. As a result of this unique reproductive physiology, bitches spontaneously ovulate only once or twice per year and ovulation can occur at any time of the year. However, there are few exceptions, such as the Tibetan Mastiff and the Basenji.[1] The interestrus interval is the time from the onset of proestrus to the subsequent onset of proestrus, and includes proestrus, estrus, diestrus and obligate anestrus periods. Proestrus is diagnosed clinically by the onset of vulvar edema and/or serosanguinous discharge, whereas estrus is defined as the onset of either behavioral signs (willingness to allow mating) or vaginal epithelial exfoliative cytology (>90% cornification). In some bitches, estrus is much less frequent or outward (visible) signs of estrus are minimal to non-existent, giving the appearance of a prolonged interestrous interval (persistent anestrus). The interestrous interval averages 31 weeks[2,3] with a typical range of 16 to 56 weeks.[3] Bitches with longer than average interestrous intervals have reduced opportunities of becoming pregnant.[4] The variation in interestrous interval length owes itself to differences in the duration of anestrus. The duration of anestrus differs between and within dog breeds indicating a genetic basis for anestrus length.[5] It is important to mention that neither the ovary nor the pituitary are quiescent during anestrus.

Factors that terminate anestrus and trigger the onset of an ensuing estrous cycle are not fully understood. In the bitch, progression from early to late anestrus is characterized by a higher amplitude and larger number of hypothalamic GnRH pulses,[6] an increase in pituitary sensitivity to GnRH,[7] and an increase in ovarian responsiveness

to LH and FSH.[7,8] Serum FSH concentrations are increased throughout much of canine anestrus while LH concentrations are low except near the end of anestrus.[9] An increase in basal plasma FSH concentration is critical for initiation of folliculogenesis in dogs.[10,11] Folliocle-stimulating hornone induces expression of LH receptors in the ovarian granulosa cells.[5] Following initial follicle recruitment, LH is progressively able to replace FSH in the support of follicular maturation.[12] In fact, supraphysiologic doses of LH alone administered to bitches in anestrus will induce follicle growth and proestrus.[9,13] It is also important to note that factors causing a decrease in opiodergic activity promote LH release and the termination of anestrus.[9]

Effect of estrus induction on pregnancy rates in bitches

Estrus induction is most successful in normal females. Its efficacy in bitches with reproductive disorders is unknown. It is important to mention that histological changes similar to involution in the bitch's endometrium are not complete until 135 days after the most recent estrous, regardless of whether the bitch was pregnant or not.[14] Chakraborty, et al.demonstrated that induction of fertile estrous cycles is diminished when induction occurs less than 4 months following the onset of the last proestrus.[4] Therefore, induction of estrus before this time may result in reduced pregnancy rates.

Gonadotropins

Both LH and FSH appear to be follicotropic in the dog as administration of pharmacologic doses of either LH or FSH alone induces estrus (Table 1).[9,15] An estrus induction protocol was established with combined dosages of FSH and LH designed to resemble the gradual increase of endogenous FSH coincidentally with the LH increase during proestrus.[15] However, this protocol was not successful (Table 1). The LH potency within purified or partially purified FSH products used may interfere with endogenous LH release.[16] Bouchard, et al. demonstrated that LH cross-reactivity from contamination of porcine-derived FSH lasts 48 hours following administration.[16] In addition, acute allergic reactions have been reported following intravenous administration of LH (5 mg) in two bitches.[17]

In addition to exogenous pituitary gonadotropins, PMSG and HMG have been used for estrus induction in bitches. The most widely studied gonadotropin for estrus induction in the dog is PMSG, with protocols ranging from daily to weekly injections using either subcutaneous or intramuscular routes of administration (Table 1). Studies using PMSG have generally been more successful for estrus induction in bitches than those using FSH. Pregnant mare serum gonadotropin is not commercially available within the U.S. except in combination with hCG (PG600®, Intervet/Schering Plough Animal Health, Millsboro, DE). This product contains 80 IU PMSG and 40 IU hCG per ml. Nickson, et al. demonstrated that a single 5-ml injection of PG600® was highly effective at inducing proestrus in bitches (17 of 19).[18] Unfortunately, the ovulation rate was poor (8 of 19), superovulation may have occurred and pregnancy rates were not reported.[18] However, others have reported 50-84% whelping rates when PMSG and hCG are given in combination to induce estrus in bitches.[19,20]

The most frequent problems encountered with PMSG arise from the unpredictability of an individual bitch's response both in the number of follicles that develop and in the potential for allergic reaction and premature luteal failure. There has been one report of an immune-mediated reaction in a Boxer bitch that received a second PMSG injection for estrus induction[21] and another case of a bitch that died unexpectedly following treatment with PMSG (2000 IU).[22] Premature luteal failure with subsequent shortening of diestrus and pregnancy loss is a frustrating sequelae of PMSG use in canids.[23-27] In one study, treatment with PMSG was followed by a progressive decline in progesterone concentrations below 1 ng/ml between 38-40 days post-estrus.[26] Histologically, luteal cells from corpora lutea formed in bitches following PMSG treatment have reticulated and vacuolated cytoplasm compared to luteal cells from corpora lutea of normal, non-fertile estrous cycles that have compact and granulated cytoplasm.[27]

Premature luteolysis of induced corpora lutea have also been reported in ewes,[28] beef cows[29]] and dairy cows.[30] In ruminants, pretreatment with a progestin prior to ovulation induction increases luteal weight[31] and secretion of progesterone.[32-35] However, bitches pretreated with megestrol acetate (2.2 mg/kg orally once daily for 8 days) before undergoing an estrus induction with PMSG (44 IU/kg intramusculary once daily for 9 days) were not prevented from undergoing premature luteolysis as progesterone values were <1 ng/ml by 50 days post-estrus in all PMSG-treated bitches.[36] It is of interest to note that such premature luteal regression appears to be independent of the presence or absence of the uterus.[36] This was demonstrated following hysterectomy of normal, nonpregnant bitches on day 4 of estrus during a non-induced cycle. Hysterectomy resulted in premature regression of the corpora lutea.[37] The authors speculated that a luteotrophic factor of uterine origin, which may be active in the normal cycle of the bitch at 24 days of diestrus may be involved in luteal maintenance.[37]

Administration of an ovulation induction agent in bitches as part of an estrus induction protocol is controversial since bitches are spontaneous ovulators and such a treatment would be unnecessary. Administration of hCG has no positive effects on ovulation rates, pregnancy rates or number of offspring per pregnancy when administered at the onset of or during estrus.[38] In fact, treatment with hCG on the first and third days of estrus significantly prolongs behavioral estrus and lowers serum progesterone concentration of day 5 of estrus.[38] Volkmann, et al. found similar results when hCG was administered to bitches after day 40 of gestation; in that following an initial increase in serum progesterone concentrations, hCG dramatically suppresed progesterone secretion.[39] Nevertheless, Wright reported that ovulation in the bitch occurs 26-30 hours following the administration of hCG[40] and many protocols for estrus induction in bitches include its use. It should be noted that intravenous dosages of 5-10 mg LH have also been recommended to induce ovulation in bitches.[41]

Estrogens

In anestrous bitches, treatment with estradiol 17-β has been shown to increase the concentration of GnRH in the hypothalamus.[42] An estrogen peak occurring approximately 30 days before the onset of estrus is believed to be required to prime the hypothalamus-pituitary-ovarian axis, causing pulsatile release of LH.[8,15] In addition, levels of mRNA encoding estrogen receptors α and β in the hypothalamus, pituitary and ovaries increase from late anestrus to proestrus in bitches.[43] Different approaches have been investigated to induce the release of LH and the formation of LH receptors in preovulatory follicles using estrogenic compounds.

Protocols using estrogens for estrus induction in bitches typically also include FSH or PMSG for folliculogenesis and hCG or LH for induction of ovulation (Table 2). Successful induction of fertile estrus in bitches has been accomplished with diethylstilbesterol (DES) in various doses with or without FSH and LH.[44-46] Using a combination of DES and FSH, pregnancy rates of 33% could be obtained immediately following parturition or following prostaglandin F2α (PGF) termination of diestrus.[16] It is important to note that successful induction of fertile estrus in bitches can also be accomplished with DES alone (Table 2).[16,44,47,48] In addition, substances with estrogenic properties (such as bis(p-acetoxyphenyl)cyclohexlidenemethane) have been used to successfully induce fertile estrus in bitches.[49] While short-term (seven days or less) oral treatment with estradiol 17-β[50] or DES[16] reportedly does not produce any side effects, long-term oral DES therapy used to treat urinary incontinence may result in alopecia[51]] and bone marrow suppression.[52]

GnRH and GnRH analogs

In the bitch, progression from early to late anestrus is characterized by a higher amplitude and larger number of GnRH pulses,[6] an increase in pituitary sensitivity GnRH.[8,53,54] Different approaches have been investigated to directly stimulate the activity of the pituitary with GnRH and GnRH analogs to induce estrus (Table 3). Pulsatile administration of GnRH at doses of 0.2-0.4 μg/kg at 90 min intervals is sufficient to obtain increases in LH similar to the endogenous pulses that normally occur at the end of proestrus.[9] However, estrus induction protocols using short-acting native GnRH or GnRH agonists are not clinically applicable due to the expense of pulsatile infusion pumps or need for hospitalization during continuous intravenous infusion.

It is important to note that increases in GnRH do not need to be pulsatile to induce estrus.[9] Constant infusion or release of a GnRH analog via a subcutaneous osmotic mini pump or implant resulted in similar estrus induction and pregnancy rates as GnRH pulsatile infusion, provided that the GnRH agonist therapy is discontinued.[9,55] Premature luteal failure resulting in a shortened diestrus with subsequent pregnancy loss has been reported with GnRH agonist therapy for estrus induction.[55-62]

Analogs of GnRH are used in human and veterinary medicine to stimulate (upregulate) as well as downregulate LH and FSH within the pituitary gland. By making molecular changes to native GnRH, more than 700 GnRH analogs have been synthesized that have an increased receptor affinity and enhanced stability.[63] High rates of fertile estrus induction required GnRH agonist administration for >8 days.[64] However, reduced efficacy of GnRH agonists occurs at high doses due to a failed or insufficient LH surge at the end of proestrus at doses of 24-48 μg/kg/day.[64] In humans, intranasal administration of a GnRH agonist is used as a painless, simple and practical method for several gynecologic conditions.[65-67] This intranasal spray (Leupron Depot, Takeda Chemical Industries, Osaka, Japan) was administered to 14 anestrous beagle bitches and produced no negative clinical effects and appeared to cause little stress to the animals.[43] Deslorelin is a D-Trp^{6}-Pro^{9}-des-Gly^{10}GnRH analog with two amino acid substitutions. Veterinary clinical applications of deslorelin in bitches were first introduced by Trigg, et al. during an investigation for a novel contraceptive, which is now commercially available in Australia (Suprelorin®, Peptech, North Ryde, NSW, Australia).[68] Preliminary investigations with this product demonstrated that it induced estrus in all anestrous bitches treated initially, which was followed by prolonged estrus suppression.[69,70] Deslorelin implants (Ovuplant®, Wyeth Animal Health, Guelph, ON, Canada) is a biodegradable, sustained release, subdermal

implant containing 2.1 mg of deslorelin, licensed for use in horses. According to label claims, Ovuplant® induces ovulation in mares within 48 h. Previous studies in dogs with this product demonstrate its reliability for inducing a rapid and synchronous estrus.[56,57,71] Also, in diestrous bitches (n=15), synchronous estrus could be induced following termination of diestrous using PGF with either a whole (2.1 mg) or a half (1.05 mg) of a deslorelin implant.[72] Treatment with PGF included starting with a low dose (50 μg/kg subcutaneously twice daily on the first day, followed by 100 μg/kg twice daily on the second day) and then the full dose (250 μg/kg subcutaenously twice daily) for 5 days.[72]

Dopamine agonists

Dopamine agonists successfully induce fertile estrus in most bitches (Table 4). Dopaminergic agonists are ergot derivatives that inhibit prolactin secretion by stimulating secretion of dopamine or suppressing secretion of serotonin.[73] Prolactin appears to play a part in canine interestrous intervals, possibly by affecting gonadotropin secretion and/or ovarian responsiveness to gonadotropins. Administration of dopamine agonists shortens the duration of anestrus[74,75] or induces estrus in cases of prolonged anestrus.[73-76] However, prolactin inhibition alone is not sufficient to terminate anestrus in bitches. This was demonstrated by treating bitches with low doses of a seratonin receptor antagonist (metergoline). Low doses of metergoline lower the plasma prolactin concentration via a serotonin-antagonistic pathway, while higher dosages also result in a dopamine-agonistic effect.[77-79] At low dosages, metergoline suppresses prolactin concentrations similar to concentrations observed with dopamine agonists (bromocriptine and cabergoline), but does not induce estrus.[78] However, at higher dosages (12.5 mg intramuscularly every three days until onset of proestrus), metergoline administration will result in estrus induction.[80] These observations indicate that the induction of the follicular phase is not initiated by only the suppression of prolactin secretion but by other dopaminergic effects.[81]

It was previously believed that prolactin inhibition was necessary for estrus induction to occur using dopamine agonists. Bitches that did not respond to dopamine agonist therapy (e.g. proestrus was not initiated) did not have a decrease in prolactin concentrations.[9] These observations suggested that an inhibition of prolactin secretion may regulate the initiation of proestrus. However, in normal cycling bitches, prolactin concentrations during late anestrus do not change prior to the onset of proestrus.[82] Then, in 2003, Beijerink, et al. demonstrated that bromocriptine shortens the interestrous interval in the bitch even when the dose is so low that it does not lower plasma prolactin concentration.[83] This suggests that dopamine agonists induce estrus with another mechanism other than via lowering plasma prolactin concentration. Kooistra, et al. reported that follicle development and resulting estrus induction with bromocriptine was associated with an increase in plasma FSH concentration without a concomitant increase in plasma LH concentration.[84] The dopamine agonist induced rise in the basal plasma FSH concentration was similar to what is observed during physiologic late anestrus.[5] In rats, the dopamine agonists and antagonists affect ovarian steroidogenesis.[85] Interestingly, in the mare, both dopamine receptors are present in the ovary[86] indicating a possible direct role for dopamine agonists. However, data are not available for dogs. It should be noted that prolonged cabergoline administration during proestrus and estrus does not affect follicular development.[87]

The effectiveness of dopamine agonists for estrus induction depends on dose, treatment duration and stage of anestrus. Beijerink, et al. demonstrated that the extent of shortening the interestrous interval by bromocriptine is dose dependent.[83] Jöchle, et al. treated 28 beagle bitches that were 4-6 months after the last estrous with cabergoline (0.005 mg/kg/day for 14 days orally) and found no difference between controls in the interestrus interval.[73] In addition, this method of estrus induction may require longer than 30 days of treatment before the onset of proestrus occurs, which is dependent upon the stage of anestrus (early versus late anestrus).[88] In contrast to this report, Cirit, et al. found no correlation between the stage of anestrus and treatment duration when cabergoline was used for estrus induction.[38]

Administration of dopamine agonists can be cost prohibitive in the United States, where these drugs are not readily available for veterinary use. Cabergoline (0.5 mg/tablet) is available as Dostinex® (Pfizer, New York, NY) and in a generic form (Par Pharmaceutical, Inc., Woodcliff Lake, NJ), but remains relatively expensive (approximately $20.00/tablet) and difficult to dose accurately in small dogs.[89] For accurate dosing in dogs, tablets can either be compounded into the appropriate-strength capsules, or a portion of the tablet can be crushed and diluted with fluid just before dosing.[89] Cirit, et al. observed that Dostinex® tablets totally and easily dissolve in distilled water at room temperature (10 μg cabergoline/ml).[38] It had previously been reported by Persiani, et al. that the relative bioavailability of tablets versus aqueous solution of cabergoline was 99% and the pharmacodynamics and relative bioavailability was not influenced by formulation (tablet versus solution).[90] Cabergoline is inactivated over time in aqueous solutions containing water, such that the cabergoline solutions should be prepared fresh daily

and used within 15 minutes of preparation.[38] However, McLean, et al. also report that cabergoline is stable for 28 days if compounded in acidic fluids (1% acetic acid solution).[89]

There are two prominent side effects with dopamine agonists: coat color changes and vomiting. Approximately 25% of bitches that received cabergoline for 14-45 days developed coat color changes beginning the second week of administration and lasting until the next coat shedding.[91] Of these, fawn-colored bitches developed a yellowish coat color while Argentine boarhounds became black spotted, mainly on their extremities.[91] In previous untreated estrous periods, these bitches had shown no coat color changes. These authors postulated that a color shift in certain hair coats of particular breeds could be mediated through inhibition of melanocyte-stimulating hormone secretion. Transient coat color changes should be considered a possible side effect when planning long-term treatment with dopaminergic agonists in dogs.[91]

Lastly, centrally acting dopamine agonists (e.g. bromocriptine) commonly induce vomiting. Vomiting was a frequent side effect (3-25% of cases) with bromocriptine or cabergoline occurring within one hour after the first treatment.[73,74,88,92-94] Vomiting tends to be a less common side effect following cabergoline when compared to bromocriptine,[73] probably because cabergoline binds more specifically to dopamine type-2 receptors in the hypothalamus and pituitary gland.[95] It is important to note that Gunay, et al.[96] did not observe any side effects of vomiting when they administered cabergoline to German Shepherds using a much higher dose of cabergoline (6 mg/kg) than the optimal effective dose (5 µg/kg/day once daily orally) as determined by dose response.[73] Concomitant treatment with metoclopramide (0.5 mg/kg) relieved the vomiting and did not change the effect of the bromocryptine.[93] Habituation to bromocriptine, beginning with lower doses initially, is reported to almost completely eliminate emesis as a side effect of treatment.[92] It should be noted that even very high doses of metergoline do not induce vomiting in bitches.[80]

In contract to bitches, cabergoline has not been shown to be effective in domestic cats for estrus induction.[73] However, administration of 25 µg/day for 5 days or 50/µg/day for 3 days of cabergoline is effective at pregnancy termination.[73]

Normal feline reproductive physiology

Domestic cats are seasonally polyestrous. Queens are long-day breeders such that a prolonged anestrus occurs during short-day length (September–January in the Northern Hemisphere)[97,98] but may cycle throughout the year in regions where natural day length exceeds 12 hours per day yearlong (e.g. Bangkok).[99] Feline proestrus (0.5 - 2 days) is not commonly observed in queens.[100,101] Estrus is defined by estrous (receptive) behavior or changes in cornified vaginal cytology secondary to increased circulating estradiol 17-β concentrations[100,102-108] and last for a week on average. It is important to note that some queens do not display obvious signs of sexual receptivity. The first estrous cycle in cats is at 6 to 12 months of age on average, but can be as early as 4 months. Cats are induced (reflex) ovulators following adequate stimulation during mating. However, as many as 60% of unpaired, unmated and unstimulated female domestic cats ovulate without external provocation.[109-111] If the queen does not ovulate, an interestrous period follows and the cycle repeats until the daylight length is shorter than 8 hours.[112] Average length of the interestrous interval is 9.0 ± 7.6 days.[18] If the queen ovulates but does not become pregnant, diestrus is shortened. Reported lengths of the interestrous interval in bred queens that ovulated but did not become pregnant are 45.0 ± 10.3 days,[18] 50.3 ± 2.7 days[26] and 61.5 ± 14.5 days.[113]

The frequent natural estrous cycles in the nonbred queen make the utilization of estrus induction in clinical practice less likely. However, follicular development is readily induced during the non-breeding season. In fact, estrus induction response rates in queens are better during the non-breeding season.[114] Tsutsui, et al. reported that quiescent feline ovaries during the non-breeding season respond more predictably to gonadotropin stimulation compared to during the breeding season when the onset of estrus is irregular in queens and hormone administration cannot be initiated on the same day for embryo transfer synchronization.[115] The ability to suppress cyclic activity prior to ovarian stimulation has improved assisted reproductive success in the domestic cat.[116] In part, this is because a quiescent ovary is more likely to respond to estrus induction treatments with a predictable number of ovulations and a lower incidence of ovarian hyperstimulation.

Effect of estrus induction on pregnancy rates in queens

Follicle-stimulating hormone

In domestic queens, estrus can be induced with FSH administered at a dose of 2 mg IM daily until the onset of estrus, which is typically in 3 to 7 days (Table 5).[114] However, inconsistent results are observed with FSH in queens due to the variability observed among batches of partially purified FSH that has LH contamination.[117] Excessive follicle number (pronounced ovarian hyperstimulation) can also result from FSH treatment, which may be

dose dependent.[118] Approximately 10 ova were ovulated when partially purified porcine FSH was administered (6 mg total).[19,20] Ovarian hyperstimulation reduces fertility in queens. Ovarian hyperstimulation is associated with increased embryo degeneration *in vitro,*[121] and possibly increased embryo degeneration *in vivo* resulting in reduced litter sizes and pregnancy rates. Embryo degeneration may result from abnormal tubal transport or excessive amounts of endogenous estrogen resulting from excessive numbers of follicles.[122,123]

Pregnant mare serum gonadotropin

Pregnant mare serum gonadotropin has interesting effects as it exhibits both LH and FSH activity and has a long half-life. In contrast to partially purified FSH, PMSG persists in circulation for at least 120 hours.[124] As a result, only one injection of PMSG is needed to induce follicular growth. This is in contrast to estrus induction with FSH that requires daily or twice daily injections for up to a week. The feline ovary is very sensitive to PMSG. However, season, age (prepubertal compared to postpubertal), and individual threshold all contribute to variation in dosage needed to induce estrus.[125] Colby found that PMSG doses of <100 IU in domestic cats would not induce estrus.[125] However, ovarian hyperstimulation was induced when a PMSG dose of 200 IU was administered to domestic queens, resulting in an average of 39.1 ova ovulated per cat.[126] Cline, et al. reported that the ovarian hyperstimulation response produced by a PMSG dose of 300-500 IU resulted in reduced pregnancy rates (Table 5).[127]

La Polt, et al. described an increase in ovarian LH receptors resulting from PMSG administration.[128] This may explain the reports of a high percentage of follicular cysts, prematurely luteinized follicles, unovulated follicles, or follicles ovulated prior to breeding following PMSG estrus induction.[129,130] Another disadvantage of PMSG is the production of anti-gonadotropin antibodies and a secondary decrease in ovarian responsiveness to stimulation if PMSG is administered too frequently.[131] However, queens will repeatedly respond to PMSG with follicle development as long as the duration between consecutive PMSG treatments is at least 6 months.[130,131]

Naloxone

Endogenous opiods (e.g. β-endorphins) act on μ-receptors within the hypothalamus, which inhibit GnRH, and subsequently LH, secretion.[132,133] Administration of naloxone, an opiod antagonist, inhibits endogenous opiodergic tone and induces estrus in queens.[134] Gonadotropin-releasing hormone and LH release is also calcium dependent. Binding of GnRH to receptors within the pituitary produces a rapid and temporary increase in intracellular calcium ions, which results in LH secretion.[135,136] Naloxone also modulates calcium entry through L-type calcium channels.[137] In patients with high endorphin concentrations, naloxone treatment induces a rapid increase in intracellular calcium, resulting in LH secretion.[134] However, pretreatment with hCG, which increases the number of LH receptors, is necessary as administration of either hCG or naloxone alone does not induce estrus in queens.[134] Following pretreatment with hCG, 0.04 mg/kg of naloxone in a 20% calcium gluconate solution administered intramuscularly once daily for 4 days beginning on the day of hCG administration resulted in an 88% and 67% ovulation and pregnancy rate, respectively, with no undesirable side effects.[134]

Effect of ovulation induction on pregnancy rates

As stated previously, queens are induced ovulators, requiring external stimulation (such as natural breeding) to stimulate the release of pituitary LH and ovulation of mature follicles.[106,108,138-142] However, queens are often sexually receptive prior to the time when ovulation can occur. Breeding too early in estrus (prior to the third or fourth day of estrus) can result in an attenuated LH secretion and ovulation failure.[143] In addition, while the LH response sufficient to induce ovulation can occur after a single mating, repeated matings may be needed to produce a maximal rise in LH. In one report, 21% of queens ovulated after a single mating, whereas 83% of queens ovulated after multiple matings.[142] For this reason, allowing a queen to mate three times per day at 4 hour intervals throughout estrus is recommended.[144]

Use of an ovulation inducing agent in conjunction with estrus induction in queens reduces the number of matings necessary and results in predictable gestation lengths. Once follicles are mature, ovulation can be induced via exogenous administration of GnRH or hCG. Protocols using GnRH and hCG vary by frequency and route of administration as well as timing of administration relative to the onset of estrus or initiation of estrus induction treatment.

Single or multiple injections of GnRH have been reported to induce ovulation in queens.[138,145] Although a single injection of 5 to 25 μg/cat of GnRH increases serum LH concentrations in estrual domestic queens, Chakraborty, et al. reported that only the 25 μg/cat dosage consistently resulted in ovulation.[138] This is in contrast to Swanson, et al. who found that domestic queens required two injections of GnRH 12 hours apart on fourth day of estrus reliably induce ovulation.[145]

Single or multiple injections of hCG will also induce ovulation in queens.[119,120,126,127,129,146,147] Dosages of hCG for inducing ovulation in domestic cats range from 25 IU to 500 IU.[119,120,126,127,129,146,147] Tsutsui, et al. used a single (250 IU) or two doses (100 IU each) of hCG to induce ovulation.[148] Donoghue, et al. found that 100 IU hCG given on the third day of estrus also yielded satisfactory ovulation rates.[149] Wildt, et al. reported that administration of 250 IU or 500 IU of hCG twice during estrus resulted in a significantly higher ovulation rates than 50 IU hCG twice during estrus.[150] However, higher doses of hCG have a detrimental effect on oocyte quality.[151,152] There was no significant difference on pregnancy rates when an hCG dose of 100 IU was compared to 200 IU.[151] The hCG can be administered intramuscularly or intravenously. A 95% ovulation rate was reported following a single intramuscular dose of hCG (100 IU) on the third day of estrus.[153] Tanaka, et al. reported a 100% ovulation rate with intravenous administration of two doses of hCG (100 IU) at 24 hour intervals between the second and fourth day of estrus.[146] The same authors reported an ovulation rate of 91.7% in queens given a single intravenous dose of hCG (250 IU).[146] Queens that ovulate have higher estradiol concentrations during estrus than non-ovulating queens.[154]

The time of ovulation following hCG administration in domestic cats is reported to be 25-27 hours.[155,156] However, Tsutsui, et al. reported ovulation occurring in 40% of cats between 15 and 20 hours after hCG administration.[115] Pregnancy rates (~33%) following intravaginal artificial insemination with fresh semen at the time of hCG administration compared to 28 hours after hCG administration were not significantly different, although the results were slightly better when the AI was done 28 h after hCG treatment.[153] Fertilization has also resulted after natural breeding or artificial insemination as late as 41 to 49 hours after hCG administration.[156,157] The duration of sperm survival in the genital tract of the queen is not known but the observation that queens inseminated at the time of hCG administration provides evidence that fresh ejaculated cat sperm can survive in the female reproductive tract for at least 38 hours.[153] Chatdarong, et al. also provided evidence that frozen-thawed cat semen can survive in the female reproductive tract for several hours after intrauterine insemination as 45% of queens became pregnant when inseminated at the time of hCG administration.[153] Artificial insemination more than 49 hours after hCG treatment does not result in fertilization presumably due to oocyte degeneration after this time.[153,157]

The timing of administration of an ovulation inducing agent relative to the administration of FSH or PMSG can affect pregnancy rates.[129,151] By increasing the time after PMSG from 80 hours compared to 72 hours, pregnancy rates are increased from 35% to 45%.[151] More recent protocols recommend extending the interval between PMSG and hCG to 84 hours.[158] It is important to note that given alone to anestrous queens, hCG is highly folliculogenic.[159] When hCG is given during estrus, it will often induce secondary follicular growth,[124,129] which can result in embryo degeneration due to impaired oviductal transport secondary to abnormally elevated estradiol concentrations[160,161] and reduce pregnancy rates.[131,162] Also, because hCG persists in circulation for at least 96 hours[124] it can stimulate the production of anti-gonadotropin antibodies, similar to the administration of repeated injections of PMSG.[131] Therefore, administration of hCG more frequently than every six months is not recommended.

Conclusion

While many methods of estrus induction exist for both canids and felids, success (induction of estrus, ovulation, pregnancy and delivery of offspring) rates vary between and within various protocols. It is important to note that the results that have been reported here are for research animals. Long-acting preparations (placental gonadotropins, GnRH analog implants) are convenient for the owner and less stressful for the patient but are associated with premature luteal failure and subsequent reduced pregnancy rates. Because none of the agents discussed above are labeled for use in the United States, cabergoline, which is licensed in Europe for estrus induction in bitches, may be the preferred agent for canine patients. Although generic tablets are available in the United States, costs remain high. Gonadotropins (FSH, PMSG) can be used successfully in both dogs and cats, whereas dopamine agonists are only effective in bitches. Before administration, clients should be counseled on the fact that these are not approved agents in the United States and their use is off-label. Knowledge of the strengths and weakness of each regimen will assist the veterinarian in making a selection that will be best suited for each owner and patient. Owner consent must be obtained prior to using any estrus induction method in client-owned animals.

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References

1. Fuller JL: Photoperioidic control of estrus in the Basenji. J Hered 1956;47:179-180.
2. Rowlands IW: Some observations on the breeding of dogs. Proc Conf Soc Study Fertil (London) 1950;2:40-55.

3. Christie DW, Bell ET: Some observations on the seasonal incidence and frequency of oestrus in breeding bitches in Britain. J Small Anim Pract 1971;12:159-167.
4. Chakraborty PK, Wildt DE, Seager SWJ: Induction of estrus and ovulation in the cat and dog. Vet Clin North Am Small Anim Pract 1982;12:85-91.
5. Okkens AC, Kooistra HS: Anoestrus in the dog: a fascinating story. Reprod Domest Anim 2006;41:291-296.
6. Tani H, Inaba T, Tamada H, et al: Increasing gonadotropin-releasing hormone release by perfused hypothalamus from early to late anestrus in the beagle bitch. Neurosci Lett 1996;207:1-4.
7. Van Haaften B, Bevers MM, Van den Brom WE, et al: Increasing sensitivity of the pituitary to GnRH from early to late anoestrus in the beagle bitch. J Reprod Fertil 1994;101:221-225.
8. Jeffcoate IA: Endocrinology of anestrous bitches. J Reprod Fertil Suppl 1993;47:69-76.
9. Concannon PW: Biology of gonadotropin secretion in adult and prepubertal female dogs. J Reprod Fertil Suppl 1993;47:3-27.
10. Kooistra HS, Okkens AC, Bevers MM, et al: Concurrent pulsatile secretion of luteinizing hormone and follicle- stimulating hormone during different phases of the estrous cycle and anestrus in beagle bitches. Biol Reprod 1999;60:65-71.
11. Onclin K, Lauwers F, Verstegen JP: FSH secretion patterns during pregnant and nonpregnant luteal periods and 24h secretion patterns in male and female dogs. J Reprod Fertil Suppl 2001;57:15-21.
12. Monniaux D, Huet C, Besnard N, et al: Follicular growth and ovarian dynamics in mammals. J Reprod Fertil Suppl 1997;51:3-23.
13. Verstegen J, Onclin K, Silva L, et al: Termination of obligate anestrus and induction of fertile ovarian cycles by administration of purified pig LH. J Reprod Fertil 1997;111:35-40.
14. Anderson AC, Simpson ME: The ovary and reproductive cycle of the dog (Beagle). Los Altos (CA): Geron-X Inc; 1973.
15. Shille VM, Thatcher MJ, Simmons KJ: Efforts to induce estrus in the bitch, using pituitary gonadotropins. J Am Vet Med Assoc 1984;184:1469-1473.
16. Bouchard G, Youngquist RS, Clark B, et al: Estrus induction in the bitch using a combination diethylstilbesterol and FSH-P. Theriogenology 1991;36:51-65.
17. Bardens JW: Hormonal therapy for ovarian and testicular dysfunction in the dog. J Am Vet Med Assoc 1971;159:1405.
18. Nickson D, Renton JP, Harvey MJA, et al: Oestrus induction in the bitch. Proc 12th Int Cong Anim Reprod 1992;4:1799-1801.
19. Scrogie NJ: The treatment of sterility in the bitch by use of gonadotrophic hormones. Vet Rec 1939;51:265-268.
20. Takeishi M, Kodoma Y, Mikami T, et al: Studies on reproduction in the dog. XI. Induction of estrus by hormonal treatment and results of the following insemination. Jap J Anim Reprod 1976;22:71-75.
21. Carter JG: Hormone treatment for nonproductive bitches. Can Vet J 1980;21:185.
22. Greenblatt RB, Pund ER: The gonadotrophins: a clinical and experimental study. South Med J 1941;36:730-742.
23. Jones GE, Boyns AR, Bell ET, et al: Immunoreactive luteinizing hormone and progesterone during pregnancy and following gonadotropin administration in beagle bitches. Acta Endocrinol 1973;72:573-581.
24. Nakao T, Yukiyo A, Fukushima S, et al: Induction of estrus in bitches with exogenous gonadotropins, and pregnancy rate and blood progesterone profiles. Jap J Vet Sci 1985;47:17-24.
25. Weilenmann R, Arnold S, Dobeli M, et al: [Estrus induction in bitches by the administration of PMSG and HCG]. Schweiz Arch Tierheilkd 1993;135:236-241.
26. Barta M, Archibald LF, Godke RA: Luteal function of induced corpora lutea in the bitch. Theriogenology 1982;18:541-549.
27. Ishihara M, Kita I, Honjo H, et al: Clinical studies on the artificial estrus of bitches. Res Bull Fac Agr Gifu Univ 1982;46:249-256.
28. Haresign W, Foster JP, Haynes NB, et al: Progesterone levels following treatment of seasonally anestrous ewes with synthetic LH-releasing hormone. J Reprod Fertil 1975;43:269-279.
29. Kessler DJ, Weston PG, Pimentel CA, et al: Dimunition of the *in vitro* response to luteinizing hormone by corpora lutea induced by gonadotropin releasing hormone treatment of postpartum suckled beef cows. J Anim Sci 1981;53:749-754.
30. Morrow DA, Roberts SJ, McEntee K, et al: Postpartum ovarian activity and uterine involution in dairy cattle. J Am Vet Med Assoc 1966;149:1596-1609.
31. Woody CO, Ginther OJ, Pope AL: Effects of exogenous progesterone on corpora lutea induced in anestrous ewes. J Anim Sci 1967;26:1113-1115.
32. Williams GL, Petersen BJ, Tilton JE: Pituitary and ovarian responses of postpartum dairy cows to progesterone priming and single or double injections of gonadotropin-releasing hormone. Theriogenology 1982;18:561-572.
33. Scheffel CE, Pratt BR, Inskeep EK: Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progesterone and gonadotropins. J Anim Sci 1982;54:830-836.
34. Ramirez-Godinez JA, Kiracofe GH, McKee RM, et al: Reducing the incidence of short estrous cycles in beef cows with Norgestomet. Theriogenology 1981;15:613-623.
35. Pratt BR, Berardinelli JG, Stevens LP, et al: Induced corpora lutea in the postpartum beef cow. I. Comparison of gonadotropin releasing hormone and human chorionic gonadotropin and effects of progestagen and estrogen. J Anim Sci 1982;54:822-829.
36. Archbald LF, Ingraham RH, Godke RA: Inability of progestogen pretreatment to prevent premature luteolysis of induced corpora lutea in the anestrus bitch. Theriogenology 1984; 21:419-426.
37. Hadley JC: The effect of serial uterine biopsies and hysterectomy on peripheral blood levels of total unconjugated oestrogen and progesterone in the bitch. J Reprod Fertil 1975;45:389-393.
38. Cirit Ü, Bacinoglu S, Cangul IT, et al: The effects of a low dose of cabergolin on induction of estrus and pregnancy rates in anestrous bitches. Anim Reprod Sci 2006;doi:10.1016/j.anireprosci.2006.09.005.
39. Volkmann DH, Kutzler MA, Wheeler R, et al: Failure of hCG to support luteal function in bitches after estrus induction using deslorelin implants. Theriogenology 2006;66:1502-1506.
40. Wright PJ: A study of the response of the ovaries of bitches to pregnant mare serum (PMS) and human chorionic gonadotrophin (HCG). Proc Int Cong Anim Reprod AI. 1972;2:1075-1079.
41. Bardens JW: Sterility in the bitch. In:Kirk RW, editor. Current veterinary therapy small animal practice. 3rd ed. Philadephia: WB Saunders; 1968. p. 681-682.
42. Kumar MS, Chen CL, Kalra SP: Distribution of luteinizing hormone releasing hormone in the canine hypothalamus: effect of castration and exogenous gonadal steroids. Am J Vet Res 1980;41:1304-1309.

43. Hatoya S, Torii R, Kumagai D, et al: Expression of estrogen receptor α and β genes in the mediobasal hypothalamus, pituitary and ovary during the canine estrous cycle. Neuroscience Lett 2003;347:131-135
44. Bouchard GF, Gross S, Ganjam VK, et al: Oestrus induction in the bitch with the synthetic oestrogen diethylstilboestrol. J Reprod Fertil Suppl 1993;47:515-516.
45. Shille VM, Thatcher M-J, Lloyd ML, et al: Gonadotrophic control of follicular development and the use of exogenous gonadotrophins for induction of oestrus and ovulation in the bitch. J Reprod Fertil Suppl 1989;39:103-113.
46. Concannon P, Lasley B, Vanderlip S: LH release, induction of oestrus and fertile ovulations in response to pulsatile administration of GnRH to anoestrous dogs. J Reprod Fert Suppl 1997;51:1-54.
47. Moses DL, Shille VM: Induction of estrus in greyhound bitches with prolonged idiopathic anestrus or will suppression of estrus after testosterone administration. J Am Vet Med Assoc 1988;192:1541-1545.
48. Hancock JL, Rowlands IW: The physiology of reproduction in the dog. Vet Rec 1949;61:771.
49. Einer-Jensen N: Induction of ovulation by bis(p-acetoxyphnyl) cyclohexylidenemethane (F6066) in the dog. Nord Vet Med 1967;19:236-239.
50. Hatoya S, Torii R, Wijewardana V, et al: Induction of fertile oestrus in bitches by an intranasal spray of gonadotrophin-releasing hormone agonist. Vet Rec 2006;158:378-379.
51. Barsanti JA, Medleau L, Latimer K. Diethylstilbesterol-induced alopecia in a dog. J Am Vet Med Assoc 1983;182:63-64.
52. Davis WM: Hapten-induced, immune-mediated thrombocytopenia in a dog. J Am Vet Med Assoc 1984;184:976-977.
54. Hull ME, Kenigsberg DJ: Gonadotropin releasing hormone: function and clinical use. Lab Manag 1987;25:51-58.
55. Concannon PW: Induction of fertile oestrus in aneostrus dogs by constant infusion of GnRH agonist. J Reprod Fert Suppl 1989;39:149-160.
56. Kutzler MA, Wheeler R, Volkmann DH. Serum deslorelin concentrations in bitches after Ovuplant® administration. Proc Annu Meet Soc Therio 2001. p. 13(abstract).
57. Kutzler MA, Wheeler R, Volkmann DH. Canine estrus induction using the GnRH agonist, deslorelin. Proc Annu Meet Eur Vet Soc Small Anim Reprod 2001. p. 147-148(abstract).
58. Cheng KW, Ngan ESW, Kang SK, et al: Transcriptional down-regulation of human gonadotropin-releasing hormone (GnRH) receptor gene by GnRH: role of protein kinase C and activating protein 1. Endocrinology 2000;141:3611-3622.
59. Garrel G, Lerrant Y, Siriostits C, et al: Evidence that gonadotropin-releasing hormone stimulates gene expression and levels of active nitric oxide synthase type I in pituitary gonadotrophs, a process altered by desensitization and, indirectly, by gonadal steroids. Endocrinology 1998;139:2163-2170.
60. Maclellan LJ, Bergfeld EG, Earl CR, et al: Influence of the luteinizing hormone-releasing hormone agonist, deslorelin, on patterns of estradiol-17β and luteinizing hormone secretion, ovarian follicular responses to superstimulation with follicle-stimulating hormone, and recovery and *in vitro* development of oocytes in heifer calves. Biol Reprod 1997;56:878-884.
61. McRae GI, Roberts BB, Worden AC, et al: Long-term reversible suppression of oestrus in bitches with nafarelin acetate, a potent LHRH agonist. J Reprod Fertil 1985;74:389-397.
62. D'Occhio MJ, Aspden WJ: Endocrine and reproductive responses of male and female cattle to agonists of gonadotrophin-releasing hormone. J Reprod Fertil Suppl 1999;54:101-114.
63. Jacobi GH, Wenderoth UK: Gonadotropin-releasing hormone analogues for prostate cancer: untoward side effects of high-dose regimens acquire a therapeutical dimension. Eur Urol 1982;8:129-134.
64. Concannon PW, Temple M, Montanez A, et al: Effects of dose and duration of continuous GnRH-agonist treatment on induction of estrus in beagle dogs: competing and concurrent up-regulation and down-regulation of LH release. Theriogenology 2006;66:1488-1496.
65. Elgendy M, Afnan M Holder R, et al: Reducing the dose of gonadotrophin-releasing hormone agonist on starting ovarian stimulation: effect on ovarian response and in-vitro fertilization outcome. Hum Reprod 1998;13:2382-2385.
66. Sizonenko PC, Reznik Y, Aubert ML: Urinary excretion of [D-Ser(t-Bu)6,Des-Gly10]GnRH ethylamide (buserelin) during therapy of central precocious puberty: a multicentre study. Acta Endocrin 1990;122:553-558.
67. Franssen AM, Kauer FM, Chadha DR, et al: Endometriosis: treatment with gonadotrophin-releasing hormone agonist Buserelin. Fertil Steril 1989;51:401-408.
68. Trigg TE, Wright PJ, Armour AF, et al: Long-term reversible desexing of male dogs and oestrus postponement of bitches, using a GnRH analogue implant. Proc Adv Dog Cat Exotic Carniv Reprod 2000. p. 21 (abstract).
69. Trigg TE, Wright PJ, Armour AF, et al: Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function in male and female domestic dogs. J Reprod Fertil Suppl 2001;57:255-261.
70. Wright PJ, Verstegen JP, Onclin K, et al: Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progestin. J Reprod Fertil Suppl 2001;57:263-268.
71. Kutzler MA, Wheeler R, Lamb S, et al: Deslorelin implant administration beneath the vulvar mucosa for the induction of synchronous estrus in bitches. Proc Annu Meet Eur Vet Soc Small Anim Reprod 2002.(abstract).
72. Volkmann DH, Kutzler MA, Wheeler R,et al: The use of deslorelin implants for the synchronization of estrous in diestrous bitches. Theriogenology 2006;66:1497-1501.
73. Jöchle W, Arbeiter K, Post K, et al: Effects on pseudopregnancy, pregnancy and interoestrous intervals of pharmacological suppression of prolactin secretion in female dogs and cats. J Reprod Fertil Suppl 1989;39:199-207.
74. Van Haaften B, Dieleman SJ, Okkens AC, et al: Induction of estrus and ovulation in dogs by treatment with PMSG and/or bromocriptine. J Reprod Fertil Suppl 1989;39:330-331.(abstract).
75. Okkens AC, Bevers MM, Dieleman SJ, et al: Shortening of the interestrous interval and the lifespan of the corpus luteum of the cyclic dog by bromocriptine treatment. Vet Q 1985;7:173-176.
76. Arbeiter K, Brass W, Ballabio R, et al: Estrus induction and fertility with cabergoline, a prolactin inhibitor, in the bitch. J Small Anim Pract 1988;29:781-788.
77. Müller EE, Locatelli V, Cella S, et al: Prolactin-lowering and -releasing drugs mechanisms of action and therapeutic applications. Drugs1983;25:399-432.
78. Okkens AC, Kooistra HS, Dieleman SJ, et al: Dopamine agonistic effects as opposed to prolactin concentrations in plasma as the influencing factor on the duration of anestrus in bitches. J Reprod Fertil Suppl 1997;51:55-58.

79. Krulich L, McCann SM, Mayfield MA: On the mode of the prolactin release-inhibiting action of the serotonin receptor blockers metergoline, methysergide and cyproheptadine. Endocrinology 1981;108:1115-1124.
80. Handaja Kusuma PS, Tainturier D: Comparison of induction of oestrus in dogs using metergoline, metergonline plus human chorionic gonadotrophin, or pregnant mares' serum gonadotrophin. J Reprod Fertil Suppl 1993;47:363-370.
81. Gobello C, Castex G, Corrada Y: Use of cabergoline to treat primary and secondary anestrus in dogs. J Am Vet Med Assoc 2002;220:1653-1654.
82. Olson PN, Bowen RA, Behrendt M, et al: Concentrations of reproductive hormones in canine serum throughout late anestrus, proestrus and estrus. Biol Reprod 1982;27:1196-1206.
83. Beijerink NJ, Dieleman SJ, Kooistra HS, et al: Low doses of bromocriptine shorten the interestrous interval in the bitch without lowering plasma prolactin concentration. Theriogenology 2003;60:1379-1386.
84. Kooistra HS, Okkens AC, Bevers MM, et al: Bromocriptine-induced premature oestrus is associated with changes in the pulsatile secretion pattern of follicle-stimulating hormone in beagle bitches. J Reprod Fertil 1999;117:387-393.
85. Mori H, Arakawa S, Ohkawa T, et al: The involvement of dopamine in regulation of steroidogeneises in rat ovarian cells. Horm Res 1994;41:36-40.
86. King SS, Campbell AG, Dille EA, et al: Dopamine receptors in equine ovarian tissues. Domest Anim Endocrinol 2005;28:405-425.
87. De Rensis F, Spattini G, Ballabio R, et al: The effect of administering a dopamine agonist (cabergoline) on follicular and luteal development during proestrus and estrus in the female greyhound. Theriogenology 2006;66:887-895.
88. Verstegen JP, Onclin K, Silva L, et al: Effect of stage of anestrus on the induction of estrus by the dopamine agonist cabergoline in dogs. Theriogenology 1999;51:597-611.
89. McLean S, Brandon S, Kirkwood R: Stability of cabergoline in fox baits in laboratory and field conditions. Wildl Res 2007;34:239-246.
90. Persiani S, Sassolas G, Piscitelli G, et al: Pharmacodynamics and relative bioavailability of cabergoline tablets vs. solution in healthy volunteers. J Pharm Sci 1994;83:1421-1424.
91. Gobello C, Castex G, Broglia G, et al: Coat colour changes associated with cabergoline administration in bitches. J Small Anim Pract 2003;44:352-354.
92. Arbeiter K, Barsch E: Possibilities of oestrus induction in the bitch with an ergoline derivate. J Vet Med 1988;35:111-117.
93. Janssens LAA: Treatment of pseudopregnancy with bromocryptine, an ergot alkaloid. Vet Rec 1986;119:172-174.
94. Zoldag L, Fekete S, Csaky I, et al: Fertile estrus induced in bitches by bromocriptine, a dopamine agonist: a clinical trial. Theriogenology 2001;55:1657-1666.
95. Rains CP, Bryson HM, Fitton A: Cabergoline. a review of its pharmacological properties and therapeutic potential in the treatment of hyperprolactinaemia and inhibition of lactation. Drugs 1995;49:255-279.
96. Gunay A, Gunay U, Soylu MK: Cabergoline applications in early and late anoestrus periods on German Shepherd dogs. Revue Méd Vét 2004;155:557-560.
97. Scott PP, Lloyd-Jacobs MA: Reduction in the anestrous period of laboratory cats by increased illumination. Nature 1959;184:2022.
98. Hurni H: Daylength and breeding in the domestic cat. Lab Anim 1981;15:229-231.
99. Shille VM, Sojka NJ: Feline reproduction. In: Ettinger SJ, Feldman EC, editors. Textbook of veterinary internal medicine. Philadelphia: WB Saunders; 1995. p. 1690-1698.
100. Shille VM, Lundstrom KE, Stabenfeldt GL: Follicular function in the domestic cat as determined by estradiol-17 beta concentrations in plasma: relation to estrous behavior and cornification of exfoliated vaginal epithelium. Biol Reprod 1979;21:953-963.
101. Stover DG, Sokolowski JH: Estrous behavior of the domestic cat. Fel Pract 1978;8:54-58.
102. Micheal RP: Sexual behavior and the vaginal cycle in the cat. Nature 1958;181:567-568.
103. Mowrer RT, Conti PA, Rossow CF: Vaginal cytology: an approach to improvement of cat breeding. Vet Med Small Anim Clin 1975;70:691-696.
104. Herron MA: Feline vaginal cytologic examination. Fel Pract 1977;3:36-39.
105. Mills JN, Valli VE, Lumsden JH: Cyclical changes of vaginal cytology in the cat. Can Vet J 1979; 20:95-101.
106. Gay VL, Johnson LM: Patterns of LH release in the ovariectomized, estrogen treated cat following sequential copulations or GnRH injections. Biol Reprod Suppl 1982;26:51(abstract).
107. Micheal RP: Estrogen-sensitive neurons and sexual behavior in female cats. Science 1962;136:322-323.
108. Wildt DE, Chan SYW, Seager SWJ, et al: Ovarian activity, circulating hormones, and sexual behavior in the cat. I. Relationships during the coitus-induced luteal phase and the estrous period without mating. Biol Reprod 1981;25:15-28.
109. Gundermuth DF, Newton L, Daels P, et al: Incidence of spontaneous ovulation in young, group-housed cats based on serum and faecal concentrations of progesterone. J Reprod Fertil Suppl 1997;51:177-184.
110. Graham LH, Byers AP, Widlt DE, et al: Natural versus chorionic gonadotropin-induced ovarian responses in the tiger assessed by fecal steroids. Biol Reprod Suppl 1996;54:114(abstract).
111. Lawler DF, Johnston SD, Hegstad RL, et al: Ovulation without cervical stimulation in domestic cats. J Reprod Fertil Suppl 1993;47:57-61.
112. Michel C: Introduction of oestrus in cat by photoperiodic manipulations and social stimuli. Lab Anim 1993;27:278-280.
113. Root MV, Johnston SD, Olson PN: Estrous length, pregnancy rate, gestation and parturition lengths, litter size, and juvenile mortality in the domestic cat. J Am Anim Hosp Assoc 1995;31:429-433.
114. Wiebe VJ, Howard JP: Pharmacologic advances in canine and feline reproduction. Top Companion Anim Med 2009;24:71-99.
115. Tsutsui T, Yamane I, Hattori I, et al: Feline embryo transfer during the non-breeding season. J Vet Med Sci 2000;62:1169-1175.
116. Pelican KM, Wildt DE, Pukazhenthi B, et al: Ovarian control for assisted reproduction in the domestic cat and wild felids. Theriogenology 2006;66:37-48.
117. Beckers JF: Isolation and use of porcine FSH to improve the quality of superovulation in cattle. Theriogenology 1987;27:213(abstract).
118. Goodrowe KL, Wildt DE: Ovarian response to human chorionic gonadotropin or gonadotropin releasing hormone in natural and induced-estrus in cats. Theriogenology 1987;27:811-817.

119. Tsutsui T, Sakai Y, Matsui Y, et al: Induced ovulation in cats using porcine pituitary gland preparation during the non-breeding season. Jpn J Vet Sci 1989;51:677-683.
120. Tsutsui T, Sato M, Kurosawa N, et al: Embryo transfer in the cat during the non-breeding season. Nippon Juigaku Zasshi 1989;51:871-877.
121. Verstegen JP, Onclin K, Silva LDM, et al: Superovulation and embryo culture *in vitro* following treatment with ultra-pure follicle-stimulating hormone in cats. J Reprod Fertil Suppl 1993;47:209-218.
122. Cline EM, Randall PA, Oliphant G: Hormone mediated oviductal influence on mouse embryo development. Fertil Steril 1973;28:766-771.
123. Stone SL, Richardson LL, Hamner CE, et al: Partial characterization of hormone-mediated inhibition of embryo development in rabbit oviduct fluid. Biol Reprod 1977;28:766-771.
124. Swanson WF, Wolfe BA, Brown JL, et al: Pharmacokinetics and ovarian-stimulatory effects of equine and human chorionic gonadotropins administrated singly and in combination in the domestic cat. Biol Reprod 1997;57:295-302.
125. Swanson WF, Bond JB, Steinetz BG, et al: Fetal and neonatal development of domestic cats produced from *in vitro* fertilization and laparascopic oviductal embryo transfer versus natural mating. Theriogenology 2001;55:371(abstract).
126. Kanda M, Oikawa H, Nakao H, et al: Early embryonic development *in vitro* and embryo transfer in the cat. J Vet Med Sci 1995;57:641-646.
127. Cline EM, Jenning LL, Sojka NJ: Breeding laboratory cats during artificially induced estrus. Lab Anim Sci 1980;30:1003-1005.
128. La Polt PS, Oikawa M, Jia X-C, et al: Gonadotropin-induced up- and down-regulation of rat ovarian LH receptor message levels during follicular growth, ovulation, and luteinization. Endocrinology 1990;126:3277-3279.
129. Wildt DE, Guthrie SC, Seager SWJ: Ovarian and behavioral cyclicity of the laboratory maintained cat. Horm Behav 1978;10:251-257.
130. Chakraborty PK, Wildt DE, Seager SWJ: Induction of ovulation and oestrus in the cat and dog. Symposium on hormone and corticoid therapy. Vet Clin North Am Small Anim Pract 1982;12:85-92.
131. Swanson WF, Horohov DW, Godke RA: Production of exogenous gonadotrophin-neutralizing immunoglobulins in cats after repeated eCG-hCG treatment and relevance for assisted reproduction in felids. J Reprod Fertil 1995;105:35-41.
132. Kalra SP, Sahu A, Kalra PS: Ageing of the neuropeptidergic signals in rats. J Reprod Fertil 1993;46:11-19.
133. Wang D, Sadee W, Quillan JM: Calmodulin binding to G protein-coupling domain of opiod receptors. J Biol Chem 1999;274:22081-22088.
134. Aiudi G, Cinone M, Sciorsci RL, et al: Induction of fertile oestrus in cats by administration of hCG and calcium-naloxone. J Reprod Fertil Suppl 2001;57:335-337.
135. Attali B, Nah SH, Vogel Z: Phorbol ester pretreatment desensitizes the inhibition of Ca^{2+} channels induced by kappa opiate, alpha 2-adrenergic, and muscarinic receptor agonist. J Neurochem 1991;57:1803-1806.
136. Ghosh BR, Wu JC, Miller WL: Gonadotropin-releasing hormone-stimulated calcium mobilization is altered in pituitary cultures from anestrous ewes. Biol Reprod 1996;54:753-760.
137. Casavola V, Guerra L, Reshkin SJ, et al: The effect of naloxone on intracellular calcium in epithelial cell system. 1st World Congress on Calcium and Vitamin D in Human Life. Rome, Italy; 1996. p. 11.
138. Chakraborty PK, Wildt DE, Seager SWJ: Serum luteinizing hormone and ovulatory response to luteinizing hormone-releasing hormone in the estrous and anestrous domestic cat. Lab Anim Sci 1979:29;338-344.
139. Concannon P, Hodgson B, Lein D: Reflex LH release in estrous cats following single and multiple copulations. Biol Reprod 1980;23:111-117.
140. Johnson LM, Gay VL: Luteinizing hormone in the cat. II. Mating-induced secretion. Endocrinology 1981;109:247-252.
141. Shille VM, Munro C, Walker Farmer S, et al: Ovarian and endocrine responses in the cat after coitus. J Reprod Fertil 1983;68:29-39.
142. Wildt DE, Seager SWJ, Chakraborty PK: Effect of copulatory stimuli on incidence of ovulation and one serum luteinizing hormone in the cat. Endocrinology 1980;107:1212-1217.
143. Goodrowe KL, Howard JG, Schmidt PM, et al: Reproductive biology of the domestic cat with special reference to endocrinology, sperm function and in-vitro fertilization. J Reprod Fertil Suppl 1989;39:73-90.
144. Cain JL. Drugs used to treat reproductive disorders. Vet Clin North Am Small Anim Pract 1998;28:395-410.
145. Kanda M, Oikawa H, Nakao H, et al: Development of *in vitro* fertilized feline embryos in a modified Earle's balanced salt solution: influence of protein supplements and culture dishes on fertilization success and blastocyst formation. J Vet Med Sci 1998;60:423-431.
146. Tanaka A, Takagi Y, Nakagawa K, et al: Artificial intravaginal insemination using fresh semen in cats. J Vet Med Sci 2000;62:1163-1167.
147. Tsutsui T, Tanaka A, Takagi Y, et al: Unilateral uterine horn insemination of fresh semen in cats. J Vet Med Sci 2000;62:1241-5.
148. Donoghue AM, Johnston LA, Goodrowe KL, et al: Influence of day of oestrus on egg viability and comparative efficieincy of in vitro fertilization in domestic cats in natural or gonadotrophin-induced oestrus. J Reprod Fertil 1993;98:85-90.
150. Wildt DE, Seager SWJ: Ovarian response in the estrual cat receiving varying dosages of hCG. Hormone Res 1978;9:144-150.
151. Goodrowe KL, Wall RJ, Wildt DE: Influence of gonadotropins on and developmental competence of *in vitro* fertilized feline follicular oocytes. Theriogenology 1988;29:250(abstract).
152. Goodrowe KL, Wall RJ, O'Brien SJO, et al: Developmental competence of domestic cat folliculate oocytes after fertilization in vitro. Biol Reprod 1988;39:355-72.
153. Chatdarong K, Axner E, Manee-In S, et al: Pregnancy in the domestic cat after vaginal or transcervical insemination with fresh and frozen semen. Theriogenology 2007 68:1326-1333.
154. Chatdarong K, Lohachit C, Kiartmanakul S, et al: Cervical patency during non-ovulatory and ovulatory estrus cycles in domestic cats. Theriogenology 2006;66:804-10.
155. Hamner CE, Jennings L, Sojka NJ: Cat (*Felis catus*) spermatozoa require capacitation. J Reprod Fertil 1970;23:477-480.
156. Sojka N, Jennings LL, Hamner CE: Artificial insemination in the cat (*Felis catus*). Lab Anim Care 1970;20:198-204.
157. Howard JG, Barone MA, Donoghue AM, et al: The effect of pre-ovulatory anesthesia on ovulation in the laparoscopically inseminated domestic cat. J Reprod Fertil 1992;96:175-86.

158. Swanson WR, Graham K, Horohov DW, et al: Ancillary follicle and secondary corpora lutea formation following exogenous gonadotropi treatment in the domestic cat and effect of passive transfer of gonadotropin-neutralizing antisera. Theriogenology 1996;45:561-572.
159. Swanson WF, Wolfe BA, Bowen JL, et al: Pharmacokinetics and ovarian stimulatory effects of exogenous gonadotropins administered singly and in combination in the domestic cat. Biol Reprod Suppl 1996;S4:189(abstract).
160. Verstegen JP, Onclin K, Silva LD, et al: Superovulation and embryo culture in vitro following treatment with ultra-pure follicle stimulating hormone in cats. J Reprod Fertil Suppl 1993;47:209-13.
161. Graham LH, Swanson WF, Brown JL: Chorionic gonadotropin administration in domestic cats causes an abnormal endocrine environment that disrupts oviductal embryo transport. Theriogenology 2000;54:1117-31.
162. Roth TL, Wolfe BA, Long JA, et al: Effects of equine chorionic gonadotropin, human chorionic gonadotropin, and laparoscopic artificial insemination on embryo, endocrine and luteal characteristics in the domestic cat. Biol Reprod 1997;57:165-171.
163. Thun R, Watson P, Jackson GL: Induction of estrus and ovulation in the bitch, using exogenous gonadotropins. Am J Vet Res 1977;38:483-486.
164. Archibald LF, Baker BA, Clooney LL, et al: A surgical method for collecting canine embryos after induction of estrus and ovulation with exogenous gonadotripins. Vet Med Small Anim Clin 1980;75:230-238.
165. Chaffaux S, Locci D, Pontois M, et al: Induction of ovarian activity in anoestrous beagle bitches. Br Vet J 1984;140:191-195.
166. Arnold S, Arnold P, Concannon PW, et al: Effects of duration of PMSG treatment on induction of estrus, pregnancy rates and the complications of hyperestrogenism in dogs. J Reprod Fertil Suppl 1989;39:115-122.
167. Allen WE: Attempted oestrus induction in four bitches using pregnant mare serum gonadotrophin. J Small Anim Pract 1982;23:223-231.
168. Renton JP, Harvey MJA, Eckersall PD: Apparent pregnancy failure following mating of the bitches at PMSG induced oestrus. Vet Rec 1984;115:383-384.
169. Wanke M, Farina J, Loza M, et al: Induction of oestrus in bitches with normal and persistent anestrus using human menopausal gonadotropin (hMG). Theriogenology 1997;47:935-942.
170. Cain JL, Cain GR, Feldman EC, et al: Use of pulsatile intravenous administration of gonadotropin-releasing hormone to induce fertile estrus in bitches. Am J Vet Res 1988;49:1993-1996.
171. Vanderlip SL, Wing AE, Felt P, et al: Ovulation induction in anestrous bitches by pulsatile administration of GnRH. Lab Anim Sci 1987;27:459-468.
172. Rota A, Mollo A, Marinelli L, et al: Evaluation of cabergoline and buserelin efficacy for oestrous induction in the bitch. Reprod Domest Anim 2003;38:440-443.
173. Inaba T, Tani H, Gonda M, et al: Induction of fertile estrus in bitches using a sustained-release formulation of a GnRH agonist (leuprolide acetate). Theriogenology 1998;49:975-982.
174. Kutzler MA: Induction and synchronization of etrus in dogs. Theriogenology 2005;64:766-775.
175. Asa CS, Bauman K, Callahan P, et al: GnRH-agonist induction of fertile estrus with either natural mating or artificial insemination, followed by birth of pups in gray wolves (*Canis lupus*). Theriogenology 2006;66:1778-1782.
176. Jeukenne P, Verstegen J: Termination of diestrus and induction of oestrus in diestrous non pregnant bitches by the prolactin antagonist cabergoline. J Reprod Fertil Suppl 1997;51:59-66.
177. Wildt DE, Kinney GM, Seager SW: Gonadotropin induced reproductive cyclicity in the domestic cat. Lab Anim Sci 1978;28:301-307.

Table 1. Published pregnancy rates following protocols using gonadotropins for estrus induction in the bitch.

Reference	N	Estrus induction protocol	Pregnancy rate
[5]	5	FSH 0.77-1.1 mg IM once	20%
[5]	4	FSH 0.077-0.11 mg to 1.23-1.78 mg IM SID for 10 d, double dose of FSH every 2 d	0%
[5]	5	FSH 0.077-0.11 mg to 1.23-1.78 mg IM every 48 hours; repeat low dose once then double dose of FSH every 2 d; LH 0.077-0.11 mg to 0.38-0.55 mg IM every 48 hours; repeat low dose four times then double dose of LH for 2 d; injections given on 1st, 3rd, 5th, 7th, 9th, 11th d	0%
[13]	16	LH 0.1 IU/kg TID for 7 d	37.5%
[19]	18	PMSG 187 MU[a] IM once[b]	50%
[24]	11	PMSG 44 IU/kg SID IM for 9 d[c]	13%[d]
[25]	14	PMSG 20 IU/kg SID IM for 5 d[e]	43%[d]
[48]	9	PMSG 200-300 IU SQ once or 100 IU SQ every 4 d for a total of 200-300 IU	11.1%
[80]	10	PMSG 33.3-71.4 IU/kg SID IM until proestrus or up to 9 d	0%
[163]	6	PMSG 15.6-35.7 IU/kg SID SC for 10 d[c]	50%
[163]	7	PMSG 15.6-35/7 IU/kg SID SC for 10 d[c]	57%
[163]	12	PMSG 1.25-2.8 IU/kg SID SC for 10 d[c]	58%
[164]	8	PMSG 44 IU/kg SID IM for 9 d[c]	60%
[164]	5	PMSG 44 IU/kg SID SQ for 9 d[f]	60%
[165]	15	PMSG 27.8-41.6 IU/kg SID IM for 10 d[c]	20%
[165]	5	PMSG 27.8-41.6 IU/kg SID IM for 10 d[g]	0%
[166]	17	PMSG 20 IU/kg SID IM for 10 d[c]	35%
[166]	6	PMSG 20 IU/kg SID IM for 5 d[h]	50%
[167]	4	PMSG 21.2-26.9 IU SC every 48 hours for a total of 5 injections (1,250 IU total)	25%[i]
[168]	3	PMSG 500 IU SID SC until onset of proestrus or up to 10 d	100%[d,j]
[169]	10	hMG 1.7 U/kg SID IM for 9 d	40%

[a] Mouse units
[b] hCG 50 MU IM at time of PMSG injection
[c] hCG 500 IU IM or SQ on 10th d of treatment
[d] Premature luteal failure
[e] hCG 500 IU IM on 5th d of treatment
[f] hCG 500 IU IM on 2nd d of estrus
[g] Gonadoliberin 0.05 mg IM on 10th d of treatment
[h] hCG 200 IU IM on 5th d of treatment
[i] Pregnant with 17 fetuses at 36 d post mating
[j] All pregnancies resorbed

Table 2. Published pregnancy rates following protocols using estrogens for estrus induction in the bitch.

Reference	N	Estrus induction protocol	Pregnancy rate
[16]	13	Diethylstilbestrol 5 mg[a] PO SID until 2nd d of proestrus; FSH 10 mg IM or 5th, 9th, and 11th d from onset of proestrus	30.8%
[44]	5	Diethylstilbestrol 5 mg/dog[a] PO SID for 6-9 d until onset of proestrus	100%
[46]	13	Diethylstilbestrol 0.1-0.2 mg/kg SID PO for 14 d; FSH 0.2-0.4 mg/kg IM on 5th, 9th, and 11th d of treatment	31%
[48]	28	Diethylstilbestrol 0.5-2.0 mg[b] every 4-5 d IM	62.5%[c]
[20]	7	Estrone 100-600 µg[d] IM every 24-48 hours (total dose 300-30,000 µg); PMSG 200-400 IU and hCG 1000 MU SQ at onset of proestrus[e]	83.7%
[50]	7	Estradiol 17-β 0.5 mg/kg PO SID for 3 d; Leuprolide 0.0036 mg[f] intranasal spray once daily following estradiol treatment until onset of proestrus or up to 14 d	71.4%
[132]	8	Bis(p-acetoxyphenyl)cyclohexylidene methans (P6066) 4 mg/kg PO SID until estrus	71.4%[g]

[a]Mongrel dogs were used but weight was not given;
[b]Airdale, foxhound and mongrel bitches, weights not given
[c]Results of breeding data only reported for 8 bitches
[d]Weight not given
[e]hCG 1000 MU and PMSG 200 IU given SQ at onset of estrus to induce ovulation
[f]Beagles were used but weight was not given;
[g]Only 7 bitches bred

Table 3. Published pregnancy rates following protocols using GnRH and GnRH analogs for estrus induction in the bitch.

Reference	N	Estrus induction protocol	Pregnancy rate
[46]	36	GnRH 0.000015-0.000500 mg/kg IV every 90 min for 7-9 d	33%
[170]	8	GnRH 0.000096-0.000139 mg/kg IV every 90 minutes for 11-13 d	87.5%
[171]	8	GnRH 0.000040-0.000430 mg/kg IV every 87 minutes for 9 d	37.5%
[172]	10	Buserelin 0.0015 mg/kg SQ TID for 11 d and 0.00075 mg/kg SQ TID for 3 d	20%
[55]	24	Lutrelin 0.0017-0.0025 mg/kg/d SQ for 12-14 d[a]	37.5%[b]
[64]	6	Lutrelin 0.048 mg/kg/d SQ for 12-14 d[a]	0%
[64]	6	Lutrelin 0.0024 mg/kg/d SQ for 12-14 d[a]	33.3%
[64]	20	Lutrelin 0.0018 mg/kg/d for SQ 12-14 d[a]	35%
[64]	6	Lutrelin 0.0012 mg/kg/d for SQ 12-14 d[a]	33.3%
[64]	18	Lutrelin 0.0006-0.0024 mg/kg/d SQ for 12-14 d[a]	88.9%
[64]	7	Lutrelin 0.0002 mg/kg/d SQ for 12-14 d[a]	57.1%
[64]	24	Lutrelin 0.0006-0.0024 mg/kg/d SQ for 7-8 d[a]	16.7%
[50]	7	Leuprolide 0.0036 mg[c] intranasal spray once daily until onset of proestrus of up to 14 d	42.9%
[173]	18	Leuprolide 0.10 mg/kg SQ once[d]	78%
[56,57]	7	Deslorelin 2.1 mg[e] SQ once	43%[b]
[71]	6	Deslorelin 2.1 mg[e] vestibular submucosa once	67%
[71]	5	Deslorelin 2.1 mg[e] vestibular submucosa once	40%
[72]	3	Deslorelin 2.1 mg vestibular submucosa once	67%
[72]	10	Deslorelin 1.05 mg vestibular submucosa once	70%
[72]	6[f]	Deslorelin 2.1 mg vestibular submucosa once	16.7%
[72]	9[f]	Deslorelin 2.1 mg vestibular submucosa once	11.1%[f]
[174]	5[h]	Deslorelin 1.5 mg IM once	60%
[175]	7[i]	Deslorelin 2.1 mg vestibular submucosa once	42.9%

[a]Via osmotic mini pump
[b]Premature luteal failure
[c]Beagles were used but weight was not given
[d]Fertirelin 0.003 mg/kg IM given on 1st d of estrus to induce ovulation
[e]Beagle bitches weighing 5.4-13.6 kg
[f]Pregnancy resulted in complete resorption and 22% of bitches developed pyometra

Table 4. Published pregnancy rates following protocols using dopamine agonists (bromocriptine, cabergoline) or seratonin antagonists (metergoline) for estrus induction in the bitch.

Reference	N	Estrus induction protocol	Pregnancy rate
[96]	48	Bromocriptine 0.3 mg/bitch for 3 d, then 0.6-2.5 mg/bitch PO SID continued 3-6 d after onset of estrus	83%
[38]	10	Cabergoline 0.005 mg/kg PO SID until 2nd d of proestrus or d 42 of treatment	60%
[38]	19	Cabergoline 0.0006 mg/kg PO SID until 2nd d of proestrus or d 42 of treatment	57.9%
[38]	8	Cabergoline 0.0006 mg/kg PO SID until 2nd d of proestrus or d 42 of treatment[a]	75%
[73]	28	Cabergoline 0.005 mg/kg PO SID for 7-10 d	93.3%
[81]	5	Cabergoline 6 mg/kg SID PO until 2nd d of proestrus	100%
[88]	5	Cabergoline 0.005 mg/kg PO SID until 3-8 d after onset of proestrus or 40 d	60%
[88]	5	Cabergoline 0.005 mg/kg PO SID until 3-8 d after onset of proestrus or 40 d	100%
[88]	5	Cabergoline 0.005 mg/kg PO SID until 3-8 d after onset of proestrus or 40 d	80%
[96]	13	Cabergoline 6 mg/kg[c] SID PO until onset of estrus or up to 14 d	84.6%
[172]	12	Cabergoline 0.005 mg/kg PO SID until progression of proestrus to estrus	83%
[176]	5	Cabergoline 0.005 mg/kg PO SID from 30 d past the LH peak until onset of prestrus	0%
[80]	12	Metergoline 0.56-1.2 mg/kg IM every 3rd d until proestrus of d 40 of treatment	75%
[80]	8	Metergoline 0.56-1.2 mg/kg IM every 3rd d until proestrus of d 40 of treatment[b]	50%

[a]hCG 500 IU administered IM on 1st and 3rd d of estrus to induce ovulation

[b]hCG 500 IU administered IM in late proestrus to induce ovulation

Table 5. Published pregnancy rates following protocols using gonadotropins for estrus induction in the queen.

Reference	N	Estrus induction protocol	Pregnancy rate
[50]	10	FSH 2.0 mg IM, then 1.0 mg IM SID until onset of estrus or the day following onset of estrus (5-6 mg total)[a]	50%[b]
[98]	3	FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6th d[c]	100%[b]
[98]	3[e]	FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6th d[c]	0%[b]
[98]	3	FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6th d[c]	100%[b]
[98]	3[e]	FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6th d[c]	0%[b]
[98]	3	FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6th d[c]	100%[b]
[98]	3[e]	FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6th d[c]	0%[b]
[177]	7	FSH 2.0 mg in saline IM SID until onset of estrus[d]	71.4%
[45]	9	PMSG 100 IU SQ once[e]	78%
[45]	20	PMSG 100 IU SQ once[f]	18%
[45]	18	PMSG 100 IU SQ once[g]	19%
[46]	10	PMSG 100 IU IM 1st d, then 50 IU SID for 2 d[h]	100%[i]
[58]	5	PMSG 100 IM 1st d, then 50 IU IM on 2nd and 3rd d[j]	100%[k]

[a]Bred to a fertile male
[b]On the basis of embryos collected
[c]hCG 250 IU IM on 6th and 7th treatment d
[d]hCG 250 IU IM on d 1 and 2 of estrus
[e]hCG 50 IU IM once 7 d after PMSG
[f]hCG 50 IU IM at time of PMSG and each subsequent d for 4-5 d (300-350 IU total)
[g]hCG 50 IU IM at time of PMSG and each subsequent d for 7-8 d (400-450 IU total)
[h]hCG 250 IU twice at 16 h intervals 7-8 d after initial PMSG injection
[i]26-65 embryos produced/queen
[j]hCG 500 IU on 7th d
[k]24-53 embryos recovered/queen

Clinical trial design and execution in small animals

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Abstract

Exceptional clinical research in veterinary medicine requires careful consideration in study design as well as consideration regarding the risks, benefits, and ethics involved. Owners must provide consent after being thoroughly informed of all aspects of the study and all options for care of their pet. Each clinical study must be well thought out with a clear, concise hypothesis. Double-blinded randomized, controlled clinical trials are one of the best tools for answering important scientific questions about veterinary care. However, many options for study design are available and can be justified as scientifically sound. Researchers must write a plan for study activities in the form of a protocol. A complete clinical study protocol should contain information on the item to be tested (drug, device, procedure), information on the control, a clear hypothesis and objectives, sample size (and how it was calculated), enrollment criteria for study animals, randomization procedures, blinding information, detailed study procedures, study timelines, data collection forms, and a data analysis and statistics plan. Clinical research in veterinary medicine is a rewarding and exciting field. Full comprehension of the components discussed will support future trial design as well as assist in the evaluation of current veterinary clinical trial literature.

Keywords: Clinical trials, cats, dogs, ethics, study design

Introduction

The veterinary scientific literature has been deficient in high quality clinical research to answer many of the important questions in the field. Veterinarians receive little training in scientific methods in veterinary school and without additional training, most lack the expertise needed to conduct or evaluate scientific research. Although many veterinarians go on to obtain further training, and many are involved in published, well-designed "bench science", it is uncommon to see well-designed, well-controlled, adequately powered clinical trials in veterinary medical publications. In order to publish more meaningful veterinary clinical research, an investigator must formulate a reasonable and testable hypothesis. They then must design and conduct a clinical trial protocol based upon measurable outcomes. Using the results of that study, an investigator must prove to the veterinary community (and possibly regulatory authorities) that the item tested (drug, food, device, technique, etc.) is efficacious for the intended use and is safe in the species for which it is intended.

Regulations and ethics in small animal clinical research

Ethics

Ethics in veterinary clinical studies are often vague and open to individual interpretation. Safety testing, patient risk/benefit analysis, using client owned animals for human research studies, and obtaining informed consent are all topics to contemplate.

Use of laboratory dogs and cats to either test the safety of a drug in normal animals or to test its effectiveness in a created laboratory model (e.g. arthritis models[1-3]) is one area of debate. Is it more ethical to use research dogs to prove safety and effectiveness, or is it more ethical to use fewer animals but be less certain of safety or efficacy prior to administering the drug to people's pets (euthanizing and/or breeding more research dogs versus risking people's beloved pets)? Safety issues in clinical trials in small animal theriogenology become a larger concern because many of the therapies to be tested do not treat a disease but are used in the process of breeding healthy animals, for example a drug to bring females in to heat.

Risk/benefit analysis is essential when designing and evaluating clinical studies. For veterinary trials, because most owners do not have health insurance for their pets, a huge benefit for clients and patients is the coverage of the cost of veterinary care. Even if the effect of the drug is uncertain, the overall effect of enrolling the pet in a clinical study may outweigh some risks because the animal receives better veterinary care. The options for many owners are to enroll their pet in a clinical trial or to euthanize because standard of care treatment for their pet's condition is not affordable. An additional consideration for the owner is the chance of their pet being enrolled into the study's control group (placebo or active comparator). Another consideration for the researcher is the ethics of removing animals from study treatment at the end of the trial. Treatment following study termination is especially important for new life-saving therapies or for therapies that dramatically increase quality of life. In these cases, an extended use or compassionate use study may be needed if the drug is not commercially available. Overall, some

studies may have clear medical benefit to the individual dog or cat, whereas, others may benefit the general population of pets and not the individual animal in a strictly medical sense.

In all cases, obtaining informed owner consent is essential to any trial in client owned animals. An informed consent document should contain all the information on study procedures, study drug(s), previous studies in this and other species, as well as any known side effects. The researcher should clearly identify the risks and benefits of study participation in simple language, approximately fifth grade level. Trained study staff should go through the consent in detail with the owner and the owner must be allowed to ask questions of the veterinarian investigator. The owner is required to sign the informed consent document prior to any study procedures being performed on their pet.

Regulations

Regulations for development of veterinary pharmaceuticals are clear. The sponsor must submit a request for an investigational new drug (INAD) to the Food and Drug Administration Center for Veterinary Medicine (FDA/CVM) prior to testing in client owned animals. Several studies must be performed to provide evidence to the FDA/CVM that the drug is both safe and efficacious for the intended use and species. The sponsor then submits the results of those evidence based studies as part of a New Animal Drug Application (NADA) and the FDA/CVM reviews the submission. If the evidence is substantial, the FDA/CVM approves the drug.

When drugs are being tested in veterinary studies in an academic setting, the regulations are more obscure. Academic (or private practice) investigators often choose to evaluate drugs licensed for use in other species (human or other veterinary) in new ways. This type of research falls in a regulatory grey area and the FDA has no specific research guidelines. The research does however fall under animal welfare laws and must be reviewed by an Institutional Animal Care and Use Committee (IACUC), if being conducted at an institution with such a committee,

Study design

All well designed studies follow a complete written protocol. A well-written protocol is especially important if more than one person is responsible for study execution. The protocol defines which animals can be enrolled and what specific procedures will be performed at each visit. Most importantly, the protocol helps maintain study procedure consistency. The protocol must be finalized prior to the first animal being enrolled in the study and ideally, not changed from that point forward (there are, of course, cases where change in the form of a protocol amendment is necessary). The complete protocol should contain information on the item to be tested (drug, device, procedure), information on the control, a clear hypothesis and objectives, sample size (and how it was calculated), enrollment criteria for study animals, randomization procedures, blinding information, detailed study procedures, study timelines, data collection forms, and a data analysis and statistics plan.

Defining the question

Investigators must define a very specific question in order that a complete protocol may be written to explain exactly how the study will enroll patients and collect the data needed to answer that specific question. The question should be formatted in such a way that it has a yes or no answer and is very specific to the population being studied and the primary measurement used to answer the question. The following is an example of the evolution of a question:

- Does deslorelin work to bring bitches into heat?
- Does the deslorelin implant work to bring bitches into heat?
- Does the deslorelin implant work at 1.05 mg to bring bitches into heat?[4]
- Does the 1.05 mg deslorelin implant bring bitches in anestrus into heat followed by ovulation (as evidenced by progesterone above 5 ng/dL) 2-4 weeks following insertion into the mucosa of the vulva as compared to a placebo group?

The question may be made more complex to obtain additional data, but a scientist should never attempt to answer too many questions with one study. If so, the focus on the primary purpose may become lost and the study then becomes a data fishing expedition. For example:

- Does the 1.05 mg deslorelin implant bring bitches in anestrus into heat as evidenced by a progesterone concentration above 5 ng/dL 2-4 weeks following insertion into the mucosa of the vulva as compared to a placebo group and is the normal inter-estrus interval maintained following use?

Now that the question is defined, it should be used it to write a hypothesis. Two hypothesis examples are below:

1) The number of anestrous bitches ovulating 2-4 weeks following implantation of a 1.05 mg deslorelin implant or placebo implant will not be different. (This is a success/fail on a per dog basis type hypothesis.)
2) There will be no difference in maximum progesterone values in the 2-4 week period following the implantation of a 1.05 mg deslorelin implant or placebo implant in anestrous bitches. (This hypothesis will measure a continuous variable, progesterone concentration, and compare the population averages among each group.)

Controls

A method of assessing a "baseline" for any measured variable is essential to demonstrating effectiveness. For this purpose, a control group is often part of the research design. A placebo-group is most common but an "active control" group (receiving another medication commonly used to treat the same disease) could also be used. It is recommended that a placebo group be used whenever possible and especially when no other treatment exists for the condition. If a treatment is available, the ethics of having a placebo group versus using the standard of care treatment as a comparator should be considered. Although animals may not be influenced by a placebo effect, owners are and subtle differences in owner behavior (especially if the owner is recording observations) may bias the study. Rarely, in a sound scientific study, is a drug compared to historical data. Historical controls are the least scientifically sound but have their place when the disease progression is very well known, disease outcome is poor, a placebo might be inappropriate, endpoints are objective, and/or each animal can be compared to its own baseline.

Outcome measurements

The measurement used to prove or disprove the hypothesis becomes the primary variable. The study is statistically designed based on this measurement. In the hypothesis 2 example above, the primary variable would be progesterone levels. Secondary variables are measurements/data collected in the study that are also of interest. To continue with the hypothesis example above, the following items could be secondary variables: luteinizing hormone levels, days to onset of heat, drug levels in plasma, other hormone levels, etc. Investigators may consider several secondary outcome measurements (variables) if the research is at a very early stage and they are unsure which variables are the best measurements for answering the question. Other variables that could also affect the outcome of the study must be considered. These "tertiary variables" (age, parity, reproductive status, breed, etc.) are mostly considered demographic data but may need to be taken into account when analyzing the results.

Statistics

Once the outcome measurements are defined and the hypothesis is written, it is time to consult a statistician. A major clinical trial should always be designed with the assistance of a statistician with experience in veterinary clinical trial analysis. If a biostatistician is not available through your place of employment (statistics or epidemiology department), there are several well-respected statisticians who work as consultants in the animal health field. It is worth every penny of expense (and is usually not that expensive in the long run) to involve a statistician in the trial design phase before crucial mistakes are made which keep investigators from obtaining meaningful study results. A statistician can help consider items that are difficult to understand for the non-statistically minded, such as continuous vs. dichotomous variables, repeated measures, "intent to treat" designs, survival, non-inferiority, blocked randomization, covariates, and stratification.

Blinding

Blinding is a condition imposed on a study meant to hide the knowledge of treatment assignment from observers. Anyone who collects data used in the analysis should be blinded. Blinding limits observer bias. A study is single blinded when the owner is unaware of the treatment group assignment. Owner blinding is helpful to eliminate subtle differences in interaction with the pet. When both investigator (and other study personnel) and owner are blinded, the term is called double-blinding. Double-blinding can eliminate investigator observational bias as well as owner bias. Triple-blinding, or masking, also blinds the person(s) performing the analysis. The maximum blinding possible should be incorporated into the design of a protocol, including the use of placebos that are indistinguishable from the investigational treatment.

Study design

Many design possibilities exist for testing a hypothesis and this paper cannot describe them all in detail. The most common is the dual-arm, randomized controlled study. Other possible designs include multi-arm (compares multiple treatments), crossover (reduces the number of patients needed because each patient receives all

treatments usually with a "washout" in between), factorial (used to test treatments alone or in combination), dose escalation (for minimal effective dose or toxicity studies), etc. Each study design has its own set of pros and cons.

Animal numbers

Once the statistician is involved, he or she can help the investigator decide how many animals will need to complete the study in order to answer the question. Calculations to derive number of animals are based on the determinant of a clinically relevant success or the limit of detection for the primary variable. For example, a 30% increase in blood flow or six months additional survival might be considered determinants of success. It is helpful to look at previous similar studies to determine what numbers (differences and standard deviation in each group) to use to estimate the results of the study being planned. Most studies are designed with a minimum of 80% power (many at 90% or 95%) and an alpha of 0.5.[5] This means that there is an 80% chance that you will detect a difference (if a difference exists), and a 5% chance that there is not a difference (if you believe a difference exists). For small pilot/exploratory studies (not intended for publication) meant to help design larger studies, the investigator may estimate animal numbers (or power if animal numbers are fixed) with the help of statistical software or online tools.[6]

Once the number of animals needed to complete the study is known, the percentage of animals that will be enrolled but then not complete the study and how many will fail to comply with the required treatments or visits should be considered. The percentage of animals predicted to complete the study can be used to determine how many animals will need to be enrolled in total. Then, the investigator must decide how many animals will need to be screened for every one animal enrolled. Be realistic when determining the failure rate at initial evaluation. Most people overestimate the number they will be able to enroll in a study. Investigators should not use enrollment challenges to justify a decrease in the number of animals targeted to complete the study. Decreasing enrollment goals may have the consequence that the study does not have the power to accept or reject the original hypothesis. In small studies with few animals, the possibility of accepting the wrong answer to the question is very real. In the scientific community, it is becoming increasingly less acceptable to include only ten animals on study due to an inability to enroll patients or due to financial concerns. The objective analysis of the numbers of animals or the amount it will cost to do the study should drive the decision to do the study at all, not if it should be done well or poorly. The advantage of conducting research in the veterinary community is that it is an inclusive group and there are likely others in theriogenology (or other specialties) who would be happy to recruit and enroll patients using the identical protocol (identical protocol is crucial). If study related cost is a factor, consider reducing the number of secondary variables to be tested or visits required instead of risking a wrong or ambiguous result due to low enrollment. The older (and sometimes newer) veterinary literature is replete with underpowered studies that enrolled only a few animals. These studies contribute very little to the profession and in some cases have come to erroneous conclusions leading many in the profession astray until later demonstrated to be incorrect.

Define the population

The study population should be defined in advance with clear, concise eligibility criteria. Specific inclusion and exclusion criteria should be created, keeping in mind that if these criteria are too strict, the study will not apply to the general population and patients may be difficult to recruit. However, if the criteria are too loose, the study may fail due to extraneous factors. Consider the following:

- Age, sex, breed effects on the study
- Will subjects be allowed to have other diseases?
- What is standard of care and how does the studied treatment fit in?
- Will study subjects be allowed other medications?
    - If not, is this applicable to the general population?
    - Do other drugs interfere with the study drug? Protein binding?
    - How might other medications affect the disease process studied?

Randomization

Randomization is one of the most effective tools for the elimination of bias from many sources. Randomization helps balance study groups and is the basis of tests for significance. Animals on the study should be assigned to groups in a random preconceived plan. This plan can be created by the study statistician or by the investigator based on the complexity of the study design. A helpful website for creating a randomization plan is: www.randomization.com.[7] Often, animals are assigned to the plan in order of enrollment in the clinical study in a way that keeps the investigator and other study staff with direct client/patient contact blinded.

Study procedures

Create a study timeline with the measurements filled in (Table 1). The first day of drug administration is traditionally Study Day 0. There should be enough leeway in the follow-up visits so that cases are not lost due to the inability of the owner to return with the pet within the given timeframe. This is especially important for long studies.

Table 1. Sample study procedures and timeline

Procedure	Day -7	Day 0 (+/- 2)	Day 14 (+/- 2)	Day 28 (+/- 4)	Day 60 (+/- 7)
Informed Consent	X				
Physical Examination	X	X	X	X	X
CBC, Chemistry, UA	X				X
Begin or End Treatment		B			E
Hormone Panel	X	X	X	X	X
Abdominal Ultrasound	X		X		X

Feasibility

Once the timeline is complete, the feasibility of the study for the investigators, study staff, and the owner is evaluated. Potential issues that may arise include fitting the study timeline into a typical workweek or workday, measurements that need to be done on weekends or after normal work hours, or time allowed to receive and evaluate the results of screening tests. In addition, the ability to recruit and enroll enough animals that fit the population criteria should be considered. If enrollment could be a problem, a multi-site study could be designed. A multi-site study requires agreement between investigators at the additional sites and an even more specific protocol (very detailed inclusion criteria, study procedures, etc.). Multi-site studies are more difficult logistically but can greatly increase enrollment on a clinical trial. All the investigators should be fully committed to running the trial for this approach to be successful. Thoughtful site selection can make or break a clinical study. The final assessment of feasibility is an evaluation of the financial aspects of the trial, especially if it is sponsored by industry or if it was designed elsewhere, to be certain the study can be conducted correctly for the money provided.

Budgeting and financing

A budget can be created from the table of procedures and events in the protocol. Recruitment and retention will be enhanced if the study pays for all veterinary care. In fact, for studies that rely on survival analysis, paid veterinary care is be essential to eliminating bias or reaching an endpoint (e.g., canine parvovirus studies). Consider adding money to compensate the investigator for time lost from clinical practice and/or money for a technician to do the majority of tasks in which direct veterinarian involvement is not necessary (discussed further below). Many studies also include client incentives for participation depending on the inconveniences compared to the benefits of the trial. Many owners will enroll their pet in a trial in exchange for free services or drugs, and others for the benefit of science, but often, an additional incentive must be offered, especially if the trial is long and requires many return visits.

Study execution

Recruitment

Study subjects may be obtained from current clinical patients in the practice database, from referring veterinarians, or by reaching out to owners directly. Consider if there will be any benefit to referring veterinarians for study patient referrals. If there will be no benefit available it may be best to advertise directly to owners. Unless you work in a private practice that is actively competing with other veterinarians for study patients, it is best to assure referring veterinarians that you will send their patients back to them once the study is completed. If you are in private practice, clinical trials can be a great way to attract new clients and increase your revenue, especially if you are involved in industry sponsored studies.

Data collection

Keep data collection to the minimum necessary to fully evaluate the endpoint variables. Create data collection forms that include space for every data point that MUST be obtained. Specific forms for each visit ensure all pertinent data are collected. The medical record is very often missing information that is essential for the study

(heart rate for example) and cannot be relied upon to obtain data for a study. In form development, it is best to use check boxes or bubbles when possible and try not to include free form text areas, especially on forms completed by owners. Limiting free text areas keeps study personnel and participants from adding information that is not needed to answer study question(s) and increasing the workload for data entry. Checklists for each visit to help the investigator and other study staff guarantee all protocol specific procedures have been completed during each study visit.

Study staff

It is worth repeating that, unless the investigator has a large amount of free time, a clinical trial coordinator or study technician should be hired. This person is usually a very detail oriented and organized veterinary nurse or assistant who will execute the details of the study including scheduling appointments, organizing and completing paperwork, calling owners, communicating with the sponsor, and generally keeping the study running smoothly. The investigator then performs only items that are specifically required to be done by the investigator (examination, diagnosis, prescriptions, etc.), decreasing his/her workload. In general, it is more cost effective for the veterinarian to see additional patients than complete study paperwork.

Protocol adherence

Stick to the protocol! Unless a true flaw is definitively identified, the protocol should be strictly followed. A reasonable change might be allowing smaller dogs to be enrolled because the investigator discovers that the tablets can be accurately cut in half (only whole tablets were anticipated). A poor reason for deviating from the protocol would be allowing one dog to be on antibiotics for a minor topical infection when your protocol specifically excludes antibiotics. If antibiotics are truly not important to the study, the protocol should be amended to allow antibiotics. A protocol amendment is a change that you make to the protocol before you initiate that change. A protocol deviation is the departure from the protocol, written after it occurred. Deviations should be avoided, if possible, to maintain the prospective nature of the study and data integrity.

Data analysis

After the data are collected and before they are given to the statistician for analysis, they should be checked for quality and accuracy. Ideally, 100% of data points are verified however, a 100% verification is often not realistic and the percentage of data checked is dependent on time and money. A plan for how to handle missing data points, deviations, skipped doses, early withdrawals, etc. should be made prior to beginning the process of including or excluding data or cases from the final analysis.

Working with industry sponsors

In general, there are two types of studies that are done in partnership with industry sponsors, investigator initiated trials and traditional safety and efficacy trials used for product registration or line extension. The level of sponsor involvement varies widely and can range from total control of the study to merely providing supplies or drug. It is absolutely essential that the role of the investigator is spelled out specifically in a contract prior to the initiation of the study. The ability of the investigator to publish the results of the study is a key issue that must be addressed prior to study initiation.

While some pharmaceutical companies enlist the most inexpensive clinics to perform clinical studies for them, most are looking for the most effective sites for clinical trials. Effective sites are those clinics that can provide excellent patient care, complete data, and are great to work with, as well as performing the studies at a reasonable (not necessary the least expensive) rate. There are very specific guidelines, such as Good Clinical Practice (GCP)[8] and International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH),[9] which have been set out by government regulators that must be followed for these types of studies. Most companies provide training to support learning these regulations and how to conduct trials in support of regulatory approvals.

Conclusion

Jerry Avorn[10] said that "The randomized controlled clinical trial is nothing less than the single most important development in the revolution of modern therapeutics, the most powerful intellectual medicine we have – the one that makes all others possible. Like Newton's laws of motion, the concept is both breathtakingly simple and enormously strong." Sound design in veterinary clinical studies is crucially important for evaluation of new therapies and techniques. Consideration of each of the discussed components can help in evaluation of published veterinary research as well as planning future research.

References

1. Intema F, DeGroot J, Elshof B, et al: The canine bilateral groove model of osteoarthritis. J Orthop Res 2008;26:1471-1477.
2. Smith GN Jr, Myers SL, Brandt KD, et al: Effect of intraarticular hyaluronan injection in experimental canine osteoarthritis. Arthritis Rheum 1998;41:976-985.
3. Botrel MA, Haak T, Legrand C, et al: Quantitative evaluation of an experimental inflammation induced with Freund's complete adjuvant in dogs. J Pharmacol Toxicol Methods 1994;32:63-71.
4. Volkmann DH, Kutzler MA, Wheeler R, et al: The use of deslorelin implants for the synchronization of estrous in diestrous bitches. Theriogenology 2006;66:1497-1501.
5. Kirby A, Gebski V, Keech AC: Determining the sample size in a clinical trial. Med J Aust 2002;177:256-257.
6. Scooernfeld DA: Statistical considerations for clinical trials and scientific experiments. Available at: http://hedwig.mgh.harvard.edu/sample_size/size.html. Accessed June 10, 2010.
7. Dallal GE: Randomization.com. Available at: http://www.randomization.com. Accessed: April 26, 2010.
8. International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products: Good clinical practice. Available at: http://www.vichsec.org/pdf/2000/Gl09_st7. Accessed June 10, 2010.
9. International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products: Home page. Available at: http://www.vichsec.org/. Accessed June 10, 2010.
10. Avorn J: Powerful medicines: the benefits, risks, and costs of prescription drugs. New York: Alfred A. Knopf; 2004. p. 53.

Non-surgical alternatives for practitioners to control reproduction in dogs and cats

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Abstract

There are numerous non-surgical methods for contraception and sterilization that have been used previously and that are currently being used. Hormonal treatments using reproductive steroid hormones (progestins or androgens) or gonadotropin-releasing hormone (GnRH) analogs result in negative feedback, effectively shutting down the hypothalamic-pituitary-gonadal axis. Immunocontraception via GnRH vaccination is also possible. The current problem with hormonal down regulation and immunocontraception is that both of these methods are reversible and therefore less desirable for large scale population control. Intratesticular injections provide a method for mechanical disruption of fertility in male dogs. There are a number of other protocols/products that have been investigated in research animals with varying degrees of success (for review, see Kutzler & Wood 2006) but for the purposes of this review, the discussion of past and current methods will focus on products were or are available to practitioners.

Keywords: Canine, contraception, feline, non-surgical, sterilization

Introduction

Over half of all United States (U.S.) households own a dog or cat.[1] However, within the U.S., there are an estimated 90 million feral or community-owned cats.[2] Although the exact figures are unknown, the Humane Society of the United States estimates that each year, between eight and ten million dogs and cats enter U.S. shelters and four to five million of these animals (predominately cats) are euthanized because there are not enough homes for them.[3] Unwanted dogs and cats are reservoirs or vectors of transmissible diseases to man and to economically valuable domestic species.[4] In Great Britain, feral cats kill approximately 100 million birds and mammals each year.[5] Free roaming dogs are a source of ecological and social problems, attacking other animals and people, causing road accidents, frightening the public, and creating fecal and urine contamination.[4]

Not all owners have their pets surgically sterilized. For those pets not intended for breeding, pet owners may reject surgical sterilization for reasons other than merely financial. In a survey in Sao Paulo, Brazil, 56.5% of owners of adopted shelter dogs were against surgical sterilization, citing compassion (58.1%), unnecessary procedure (11.4%), cost (9.5%), and behavior change (4.8%) as reasons against this method of limiting pet reproduction.[6] In addition, when considering feral cat and dog populations where permanent sterilization is desired, surgical methods can be too time consuming and expensive to be performed on a large-scale operation. Dr. Julie Levy, Director of the University of Florida Shelter Medicine program and founder of Operation Catnip (a high-volume spay/neuter clinic that has sterilized more than 40,000 feral cats since 1994) reported that "the cats were reproducing faster than we could sterilize them".[7,8] Despite the immense effort of Dr. Levy and others, only about 2% of the U.S. feral and community-owned cats are neutered (compared to an 85% sterilization rate for pet cats)[2] and the reality is that at least 75% of feral cats need to be sterilized in order to prevent population growth.[9] For the past decade, the Alliance for Contraception in Cats and Dogs (ACC&D) has pushed for a nonsurgical sterilant that "would let us reach far more animals with the same resources", says Joyce Briggs, ACC&D president.[7]

There are numerous non-surgical methods for contraception and sterilization that have been used previously and that are currently being used. Hormonal treatments using reproductive steroid hormones (progestins or androgens) or GnRH analogs result in negative feedback, effectively shutting down the hypothalamic-pituitary-gonadal axis. Immunocontraception via GnRH vaccination is also possible. The current problem with hormonal down regulation and immunocontraception is that both of these methods are reversible and therefore less desirable for large scale population control. Intratesticular injections provide a method for mechanical disruption of fertility in male dogs. There are a number of other protocols/products that have been investigated in research animals with varying degrees of success[10] but for the purposes of this review, the discussion of past and current methods will focus on products that were or are available to practitioners.

Hormonal down-regulation

Hormonal down-regulation is a useful method for achieving reversible contraception. Commercially-available options in the U.S. containing reproductive steroid hormones have declined over the last decade, although most are still available through veterinary compounding pharmacies. It is also important to note that administration of steroid hormones or GnRH analogs to pregnant dogs or cats are likely to result in fetal urogenital malformations

(e.g. hypospadias in males, masculinization in females) or abortion, respectively. Practitioners should first confirm that patients are not pregnant or be ready to accept the consequences.

Androgens have predictable effects for reversible short-term estrus suppression in female pets.[11] Mibolerone (formerly sold under the name of Cheque® Drops, Upjohn) is a synthetic androgen that was approved for use in dogs in the United States for estrus suppression.[12,13] The dose for mibolerone varies in bitches depending on body weight and breed.[14] For bitches up to 12 kg, the mibolerone dosage is 30 μg/day. For bitches 12-23 kg, the mibolerone dosage is 60 μg/day. For bitches 23-45 kg, the mibolerone dosage is 120 μg/day. For bitches over 45 kg, the mibolerone dosage is 180 μg/day. Any German Shepherd Dog or any Alsatian-derived mixed breed should receive the maximum daily dosage (180 μg/day). The reason for the higher dosage requirement within Alsatian lineage is unknown.[15] If daily treatment is initiated at least 30 days prior to the onset of proestrus, estrus can be postponed for up to two years. Following cessation of the daily treatment, return to estrus will occur within 70 days on average (range 1-7 months).[12] Continuous treatment up to five years has been demonstrated but it is generally not recommended to treat continuously for more that 24 months. The most common side effect reported in dogs is clitoral hypertrophy and vaginitis.[13,16] Other side effects include increased body odor, urinary incontinence and spraying, mounting behavior, cervical dermis thickening and epiphora.[12,14,16] Mibolerone is contraindicated for use in Bedlington terriers due to an increased risk of hepatic dysfunction and in patients with androgen-responsive neoplasias. Mibolerone has been used off-label for estrus suppression in cats at an oral dosage of 50 μg/day/queen.[13] Lower doses do not suppress estrus in queens. Hepatic dysfunction has been observed in queens at doses of 60 μg/day with mortality ensuing at doses of 120 μg/day.[14,16] Another important consideration if using mibolerone in queens is that the side effects of cervical skin thickening and clitoral hypertrophy did not resolve after drug withdrawal.[13]

Progestin administration is the most commonly used method of medical estrus suppression.[11] However, there has not been or will there be any universally safe or effective progestin. The most common progestin prescribed,[17] megestrol acetate, is a synthetic progestin that is a tasteless, odorless crystalline powder and is rapidly metabolized when given orally.[14] Megestrol acetate (Ovaban®, Intervet/Schering Plough Animal Health, Millsboro, DE) was approved in the U.S. for use in dogs and cats. The efficacy of megestrol acetate at estrus suppression has been extensively evaluated in female dogs. When given at a daily dose of 2.2 mg/kg body weight orally for eight days beginning in early proestrus, estrus was suppressed in 92% of cases.[18] Pyometra, a reported side effect of megestrol acetate treatment, developed in 0.8% of treated bitches.[18] For anestrous bitches, a lower dose of megestrol acetate (0.55 mg/kg/d orally) can be administered for prolonged periods of estrus suppression. However, prolonged megestrol acetate treatment in dogs may also cause increased appetite leading to weight gain, lethargy or restlessness,[14,16] marked mammary stimulation with hyperplastic and/or neoplastic changes and clinical and pathologic changes typical of diabetes mellitus.[19,20]

Megestrol acetate is also effective at suppressing estrus in queens when given a dose of 5 mg/cat orally for three to five days and then a dose of 2.5 to 5 mg/cat orally once weekly.[14,16,17] Prolonged use of megestrol acetate in queens causes similar side effects as those reported in bitches (weight gain, lethargy or restlessness, marked mammary stimulation with hyperplastic and/or neoplastic changes and clinical and pathologic changes typical of diabetes mellitus.[14,21,22] In addition, megestrol acetate can induce profound adrenocortical suppression, adrenal atrophy and iatrogenic Addison's syndrome in cats within one to two weeks even at the low, once weekly doses.[23] Ovulation can also be induced in queens following progestin administration.

Medroxyprogesterone acetate (Promone®, Upjohn) is a long acting injectable progestin that was also labeled for estrus suppression in the bitch and queen but used to a more limited extent compared to megestrol acetate due to a higher incidence of side effects, especially in cats.[24] The prevalence of uterine lesions identified by histopathology following an ovariohysterectomy was 45% for bitches treated with medroxyprogesterone acetate for estrus suppression compared to 5% for untreated animals.[25] In addition to uterine lesions, subcutaneous administration of medroxyprogesterone acetate in dogs has resulted in clinical signs consistent with adrenocortical suppression (e.g. alopecia, hair discoloration, thinning of the skin and mobilization of subcutaneous fat).[26] Medroxyprogesterone acetate is no longer available on the veterinary market but human generic and brand name sources (Depo-Provera®, Pfizer, New York, NY) are still used by small animal veterinarians in an extra-label manner.

Other synthetic progestins are currently available for estrus suppression in dogs and cats. Proligestone (14α,17α-propylidene-dioxy progesterone) is a unique progestin with progestational activity weaker than other synthetic progestins.[27] Proligestone is commercially manufactured in Europe (Delvosteron®, Intervet/Schering Plough Animal Health, Milton Keynes, Buckinghamshire, UK) and is labeled as an injectable canine contraceptive. The manufacturer claims that it is safe to use for prevention, delay or suppression of estrus when given to female

dogs (10-30 mg/kg SQ) and repeated at three and seven months following the initial injection.[28] This drug can be given to female cats at a dose of 1 ml subcutaneously for estrus suppression for about 6.5 months.[28] In clinical trials, this regimen did not promote development of uterine disease or mammary tumors.[27] In addition, implants containing generic levo-norgestrel or human-labeled levo-norgestrel (Norplant®, Wyeth-Ayerst) have contraceptive efficacy in female cats, but not dogs.[29]

Gonadotropin-releasing hormone analogs can also be used to down-regulate the hypothalamic-pituitary-gonadal axis and have fewer side effects and a longer duration of efficacy when compared to reproductive steroid hormones. GnRH is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2) hypothalamic hormone that acts upon GnRH receptors in the anterior pituitary to regulate the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). The endogenous hormone is released in a pulsatile manner and has a short half-life of two to five minutes due to rapid cleavage by proteases.[30,31] Sustained exposure to GnRH reduces LH and FSH secretion by GnRH receptor down-regulation, internalization and G-protein signal uncoupling. GnRH analogs (leuprolide, lutrelin, deslorelin) have been developed in long-term release formulations to reversibly suppress reproductive function in male and female dogs for periods exceeding one year.[32-37] Although use of GnRH analogs in dogs as contraceptives has been demonstrated to be safe, little is known regarding the effects after a lifetime of use. It should be noted that there is evidence that GnRH analogs used in the treatment of human prostate cancer have been associated with cardiac side effects.[38]

Several studies have examined the use of deslorelin as a male contraceptive in dogs. Biocompatible subcutaneous implants containing 4.7 mg of deslorelin acetate (Suprelorin®, Peptech Animal Health, North Ryde, NSW, Australia)) became commercially available for male dogs in Australia and New Zealand in 2004 and are labeled for contraception, treatment of benign prostatic hyperplasia and unacceptable male behavior with a 98% efficacy for at least six months.[39] The same implant has been distributed in Europe through Virbac since 2008. Serum testosterone concentrations decreased on average to less than 1 ng/mL by day 17 after implant administration and remained low for up to 2.7 years.[36,40,41] The threshold concentration of deslorelin necessary for suppressing spermatogenesis in male dogs is >0.25 mg/kg of body weight.[36] The duration of the inhibitory effect appears to be dose related, and even at the same dose, it varies greatly between individuals.[36] Restoration to initial conditions (scrotal circumference, serum testosterone concentrations, semen quality, fertility as well as testicular and prostatic histology) occurred in dogs following recovery from treatment.[36,40,42] Multiple serial implant administration in males did not cause adverse effects or diminished efficacy. Dogs that have been re-implanted for four consecutive doses at six-month intervals with the 4.7 mg deslorelin implant returned to normal steroidogenesis after cessation of treatment.[43] In 2007, Peptech Animal Health received approval in Australia for a twelve month Suprelorin® for use in male dogs that contains 9.4 mg of deslorelin acetate. Although an extra-label application, when Suprelorin® was administered to anestrous bitches, an infertile ovulatory estrus was induced four to eight days after implantation due to the initial stimulatory effect of the agonist, which was followed by prolonged interestrous intervals up to 27 months.[36] To prevent estrus induction, Suprelorin® implant administration in anestrous bitches should follow seven days of oral exogenous progestin treatment (e.g. megestrol acetate at a dose of 2 mg/kg/day).[32,44,45]

Gonazon® (Intervet/Schering-Plough Animal Health), another GnRH analog, subcutaneous implant containing 18 μg of 41% azagly-nafarelin, is labeled for twelve month estrus suppression in bitches. The threshold concentration of nafarelin necessary for estrus suppression in female dogs is <16 μg/day.[44] However, when Gonazon® was administered to anestrous bitches, estrus was induced in the month following implant insertion similar to Suprelorin®. Following implant removal at one year after insertion, a normal estrus occurred within 12-129 days followed by normal corpora lutea function as determined by serum progesterone concentrations.[46] Despite receiving regulatory approval in Europe in 2008, it is not currently commercially available.[47]

Although not a veterinary product, depot forms of leuprolide acetate (Lupron®;Abbott Laboratories, Abbott Park, IL) are available and administration of a single subcutaneous injection (1 mg/kg) to intact male dogs decreases ejaculate volume, increases morphologically abnormal spermatozoa and significantly decreases serum testosterone and LH concentrations for six weeks. Return to normal spermatogenesis occurred twenty weeks after treatment.[48] Lupron® is also effective in male cats.

Immunocontraception

Over the past decade, vaccines have been developed that suppress fertility by targeting GnRH. The physiologic effects of antibody titers against GnRH include suppression of reproductive behavior in both males and females, suppression of synthesis and secretion of gonadotropins and steroid hormones, gonadal atrophy and the associated arrest of gametogenesis.[49,50] In addition, reduction in GnRH concentrations by generation of anti-GnRH antibodies has been demonstrated to be safe and not associated with any undesirable side effects. However, development of GnRH vaccines for immunocontraception is problematic for several reasons. Native GnRH is not

naturally immunogenic because it is a small decapeptide hormone that is well conserved throughout all mammalian species. Under normal conditions, GnRH is recognized by the immune system as self (allogenic). Subsequently, administration of a vaccine derived from native GnRH results in no antibody production or a short-lived, weak response because the animal is tolerant to its own hormones. However, when GnRH is altered in a way that induces recognition of itself as a foreign material, such as coupling it with another molecule with many antigenic determinants, an IgG response will occur.[51]

In 2005, a GnRH vaccine (Canine Gonadotropin Releasing Factor Immunotherapeutic®, Pfizer Animal Health, New York, NY) for male dogs received FDA approval in the U.S. for the semi-annual treatment of benign prostatic hyperplasia. Both serum testosterone concentrations and prostate gland size decreased following vaccination and no significant systemic reactions or adverse events were observed post-vaccination. This vaccine has not been manufactured since 2008 due to low product sales but limited manufacturing may be available in the future.[52] Although this vaccine is effective for up to one year at suppressing estrus in female cats (clinical observations by author), for the purposes of controlling feral dog and cat populations, an effective immunocontraceptive should have at least a three-year duration of effect through a single treatment that induces a high (over 95%) response level in treated animals with few or manageable negative side effects.[8] In 2009, GonaCon™ was registered by the U.S. Environmental Protection Agency (#56228-40) for use with female white-tailed deer.[8] This vaccine has been studied in squirrels, swine, wild horses, white-tailed deer and other species. In swine and deer, infertility has lasted up to five years after a single vaccination. However, efficacy is not 100%. In one study of white-tailed deer, a single injection of GonaCon™ was found to be 88% effective for the first year after vaccination and 47% effective the second year after vaccination. Levy and colleagues found that a single injection of GonaCon™ contracepted male and female cats for up to five years, although the effect was diminished over time.[7] A single injection prevented pregnancy in 87%, 67%, 54%, 33% and 27% of queens for the first-fifth years after vaccination, respectively.[8] However, at 24 months after vaccination, 6/20 (30%) of queens still had palpable (non-painful) injection site granulomas.[8] Preliminary research in male dogs reviewed even more severe injection site reactions (relatively soon after injection) with painful draining tracts resulting at the injection sites.[8]

Intratesticular injections

Intratesticular injections have been investigated as a method of inducing aspermatogenic orchitis and male contraception for more than five decades.[53] In 2003, the FDA approved Neutersol Injectable Solution® (Addison Laboratories, Fayette, MO), a zinc gluconate solution neutralized to a pH of 7 by arginine, for use in puppies three to ten months of age with testes measuring 10 to 27 mm in width,[54,55] but it has been used off-label in younger puppies and large adult dogs as well.[56,57] The procedure for intratesticular injection involves inserting a needle from the caudal pole of the testis and gently pushing it towards the other pole,[58] depositing a predetermined amount of zinc solution homogenously as far as possible through the tissue. Zinc is considered to be nonmutagenic, noncarcinogenic, and nonteratogenic.[59,60] However in high concentrations, zinc inhibits the division and replication of germ cells and causes fragmentation of the cellular membrane and nucleus.[61-63] Histopathologic findings within two and a half months of injection demonstrate: lack of seminiferous germ cells, morphological changes to Sertoli and Leydig cells, atrophy of seminiferous tubules, and impairment of spermatogenesis.[57,64]

Proper injection technique is critical when zinc gluconate is administered intratesticularly because leakage or injection into non-target tissues can result in severe tissue damage. In a small clinical study consisting of 103 male dogs of various sizes and ages, necrotizing injection site reactions occurred in four animals (3.9%), which were all large mature dogs that had received a dose at the upper end of the label range (3 dogs received 0.8 mL/testicle and 1 dog received 1.0 mL/testicle).[56] Although this complication rate is similar to what has been reported following surgical castration, these reactions were more severe. In a larger field trial conducted in Mexico with 10,000 adult pet dogs, a similar complication rate (2.5%) was reported.[65,66]

The ease of use and cultural acceptance of a non-surgical option makes Neutersol Injectable Solution® a valuable option for large-scale use, particularly in remote locations lacking sophisticated clinical facilities or skilled surgeons and staff. However, the expense of the commercial product was one reason limiting its use in large sterilization campaigns. In addition, in 2005, for business reasons that had nothing to do with the drug's safety and efficacy or with consumer demand, Neutersol Injectable Solution® was no longer in production.[67,68] However, a product basically identical to Neutersol®, called EsterilSol™ (Ark Sciences, LLC, Baltimore, MD) has been available to private-practitioners, government programs, and non-governmental organizations in Mexico since 2008 and is available for limited use outside Mexico.

Conclusion

For permanent irreversible fertility control with few side effects, surgical sterilization has yet to be equaled. Yet with millions of companion animals euthanized each year, surgical sterilization can hardly be considered the gold standard for managing dog and cat populations in the U.S.[68] Despite the obvious need for alternatives to surgical sterilization, most small animal veterinarians feel that surgical sterilization can adequately manage dog and cat over population; whereas 79% of shelter veterinarians cited a need for nonsurgical sterilants.[69] According to Dr. Julie Dinnage, Executive Director of the Association of Shelter Veterinarians, the usefulness of a nonsurgical sterilant would vary by region (e.g. stray dogs are more of a problem on some American Indian reservations and in developing countries; whereas large populations of feral cats are more of a problem in other areas.[68]

The most effective method for curtailing population in a litter-bearing species, such as the dog and cat, is to sterilize the female, which is where research should be focused. In addition, since studies have estimated the average lifespan of feral cats in the U.S. at not much more than three years and 75% of feral kittens died within the first six months[2] a non-surgical sterilant effective for at least three years would significantly reduce the number of unwanted dog and cats. It is clear that no single method of reproduction control (surgical or nonsurgical) will be a panacea for dog and cat overpopulation. The more tools we have, the better positioned we will be address this problem nationally as well as internationally. Lack of funding and interest by pharmaceutical companies has historically slowed development of non-surgical sterilants, which is changing thanks to a U.S. billionaire named Gary Michelson, who has personally provided $75 million for the development of a single-use, permanent, nonsurgical sterilant for dogs and cats.[7] Through generous funding opportunities available through the Found Animals Foundation with the Michelson prizes and grants, the reality of non-surgical sterilization and an end to dog and cat overpopulation finally may be within reach.

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References

1. Olson PN, Moulton C: Pet (dog and cat) overpopulation in the United States. J Reprod Fertil Suppl 1993;47:433-438.
2. Nutter FB, Levine JF, Stoskopf MK: Reproductive capacity of free-roaming domestic cats and kitten survival rate. J Am Vet Med Assoc 2004;225:1399-1402.
3. The Humane Society of the United States: The crisis of pet overpopulation. Available at: http://hsus.org/pets/issues_affecting_our_pets/pet_overpopulation_and_ownership_statistics/the_crisis_of_pet_overpopulation.html. Accessed: January 23, 2006.
4. Dinnage J: JAVMA News: Nonsurgical alternatives explored as possible answer to dog and cat overpopulation. J Am Vet Med Assoc 2007; 230:168-170.
5. May RM: Control of feline delinquency. Nature 1988;332:392-393.
6. Soto FR, Ferreira F, Pinheiro SR, et al: Adoption of shelter dogs in a Brazilian community: assessing the caretaker profile. J Appl Anim Welf Sci 2005;8:105-116.
7. Grimm, D: A cure for euthanasia? Science 2009;325:1490-1493.
8. Alliance for Contraception in Cats and Dogs: Immunocontraceptive approaches for sterilization of dogs and cats. ACC&D Scientific Think Tank, November 10-21, 2009, Roanoke, Virginia. www.acc-d.org. Accessed: May 10, 2010.
9. Andersen MC, Martin BJ, Roemer GW: Use of matrix population models to estimate the efficacy of euthanasia versus trap-neuter-return for management of free-roaming cats. J Am Vet Med Assoc 2004;225:1871-1876.
10. Kutzler M, Wood A: Non-surgical methods of contraception and sterilization. Theriogenology 2006;66:514-525.
11. Wiebe VJ, Howard JP: Pharmacologic advances in canine and feline reproduction. Top Compan Anim Med 2009;24:71-99.
12. Johnston SD, Root Kustritz MV, Olson PNS: Canine and feline theriogenology. Philadelphia: Saunders; 2001.
13. Burke TJ, Reynolds HA, Sokolowski JH: A 280-day tolerance-efficacy study with mibolerone for suppression of estrus in the cat. Am J Vet Res 1977;38:469-477.
14. Plumb DC: Veterinary drug handbook.4th ed. Ames: Iowa State University Press; 2002.
15. Sokolowski JH: The evaluation of efficacy of mibolerone for estrus prevention in the bitch. Proc Cheque Canine Contracept B 1978;24-30.
16. Burke TJ: Pharmacologic control of estrus in the bitch and queen. Vet Clin North Am Small Anim Pract 1982;12:79-84.
17. Concannon P: Estrus suppression in the bitch. In: Bonagura JD, Twedt DC, editors. Kirk's current veterinary therapy XIV. Philadelphia: Saunders; 2008. p. 1024-1030.
18. Burke TJ, Reynolds HA: Megestrol acetate for estrus postponement in the bitch. J Am Vet Med Assoc 1975;167:285-287.
19. Food and Drug Administration (FDA): Introduction to total drug quality. Dept of Health, Education, and Welfare (DHEW) Publication #74-3006; 1973.
20. Goldenthal ET: Current views on safety evaluation of drugs. FDA Papers 1968;2:13-18.
21. Hayden DW, Barnes DM, Johnson KH: Morphologic changes in the mammary gland of megestrol acetate treated and untreated cats: a retrospective study. Vet Pathol 1989;26:104-113.
22. Peterson ME: Effects of megestrol acetate on glucose tolerance and growth hormone secretion in the cat. Res Vet Sci 1987;42:354-357.
23. Plumb DC: Veterinary drug handbook. White Bear Lake (MN): PharmaVet Publishing; 1991. p. 284.
24. Jackson EKM: Contraception in the dog and cat. Br Vet J 1984;140:132-137.
25. Von Berky AG, Townsend WL: The relationship between the prevalence of uterine lesions and the use of medroxyprogesterone acetate for canine population control. Aust Vet J 1993;70:249-250.

26. Nelson M: After-effect of injection. Vet Rec 1977;100:78.
27. Van Os JL, Van Laar PH, Oldenkamp EP, et al: Oestrus control and the incidence of mammary nodules in bitches, a clinical study with two progestogens. Vet Q 1981;3:46-56.
28. Evans JM, Sutton DJ: The use of hormones, especially progestagens, to control oestrus in bitches. J Reprod Fertil 1989;39:163-173.
29. Baldwin CJ, Peter AT, Bosu WT, et al: The contraceptive effects of levonorgestrel in the domestic cat. Lab Anim Sci 1994;44:261-264.
30. Hull ME, Kenigsberg DJ. Gonadotropin releasing hormone: function and clinical use. Lab Manag 1987;25:51-58.
31. Jiang GC, Stalewski J, Galyean R, et al: GnRH antagonists: a new generation of long acting analogues incorporating para-ureido-phenylalanines at position 5 and 6. J Med Chem 2001:44,453-467.
32. McRae GI, Roberts BB, Worden AC, et al: Long-term reversible suppression of oestrus in bitches with nafarelin acetate, a potent LHRH agonist. J Reprod Fertil 1985;74:389-397.
33. Lacoste D, Dube D, Trudel C, et al. Normal gonadal functions and fertility after 23 months of treatment to prepubertal male and female dogs with the GnRH agonist [D-Trp-6, des-Gly-NH2-10] GnRH ethylamide. J Androl 1989;10:456-465.
34. Vickery BH, McRae GI, Goodpasture JC, et al: Use of potent LHRH analogues for chronic contraception and pregnancy termination. J Reprod Fertil 1989;39:175-87.
35. Inaba T, Umerhara T, Mori J, et al. Reversible suppression of pituitary testicular function by a sustained release formulation of GnRH agonist (leuprolide acetate) in dogs. Theriogenology. 1996;46:671-677.
36. Trigg TE, Wright PJ, Armour AF, et al: Use of a GnRH analogue implant to produce reversible long-term suppression of reproductive function in male and female domestic dogs. J Reprod Fertil Suppl 2001;57:255-261.
37. Corrada Y, Hermo G, Arias D, et al: Use of the GnRH antagonist, acyline, on pregnancy termination in the bitch. Proc World Small Anim Vet Assoc Cong 2005 [abstract].
38. Skinner DC, Albertson AJ, Navratil A, et al. Effects of gonadotrophin-releasing hormone outside the hypothalamic-pituitary-reproductive axis. J Neuroendocrinol 2009;21:282-292.
39. Herbert CA, TriggTE: Applications of GnRH in the control and management of fertility in female animals. Anim Reprod Sci 2005;88:141-153.
40. Junaidi A, Williamson PE, Cummins JM, et al: Use of a new drug delivery formulation of the gonadotrophin-releasing hormone analogue deslorelin for reversible long-term contraception in male dogs. Reprod Fertil Dev 2003;15:317-322.
41. Ponglowhapan S, Lohachit C, Swangchanuthai T, et al: The effect the GnRH agonist deslorelin on prostatic volume in dogs. Proc Annu Meet Eur Vet Soc Small Anim Reprod 2002. p. 150[abstract].
42. Dube D, Assaf A, Pelletier G, et al: Morphological study of the effects of an GnRH agonist on the canine testis after 4 months of treatment and recovery. Acta Endocrinol (Copenh) 1987;116:413-417.
43. Trigg TE, Doyle AG, Walsh JD, et al: Advances in the use of the GnRH agonist deslorelin in control of reproduction. Proc Int Symp Canine Feline Reprod 2004. p. 49-51[abstract].
44. Rubion S, Desmoulins PO, Riviere-Godet E, et al: Treatment with a subcutaneous GnRH agonist prevents puberty in bitches. Proc Int Symp Canine Feline Reprod 2004. p. 56-58[abstract].
45. Wright PJ, Verstegen JP, Onclin K, et al: Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progestin. J Reprod Fertil Suppl 2001;57:263-268.
46. Rubion S, Guerin C, Rivieri-Godet E, et al: Treatment with a subcutaneous GnRH agonist containing devise suppresses FSH and ovarian function in bitches. Reprod Domest Anim 2003;79:355[abstract].
47. Driancourt MA,Rubion S. Desmoulius PO, et al: Treatment with a subcutaneous GnRH-agonist containing implant reversibly prevents bitches and queens from displaying puberty, heat and ovulation. Proc Int Symp Non-Surg Contracept Pet Popul Control, 2006. http://www.acc-d.org/2006%20Symposium%20Docs/2Driancourt.pdf. Accessed May 10, 2010.
48. Inaba T, Tani H, Gonda M, et al: Induction of fertile estrus in bitches using a sustained-release formulation of a GnRH agonist (leuprolide acetate). Theriogenology 1998;49:975-982.
49. Ladd A, Tsong YY, Walfield AM, et al: Development of an antifertility vaccine for pets based on active immunization against luteinizing hormone-releasing hormone. Biol Reprod 1994;51:1076-1083.
50. Jung MJ, Moon YC, Cho IH, et al: Induction of castration by immunization of male dogs with recombinant gonadotropin-releasing hormone (GnRH)-canine distemper virus (CDV) T helper cell epitope p35. J Vet Sci 2005;6:21-24.
51. Mitchison WA: The carrier effect in the secondary response to hapten protein conjugate. Eur J Immunol 1971;1:10-18.
52. Russo, S: Personal communication between author and Dr. Suzanne Russo, Pfizer Animal Health, April 28, 2010.
53. Freund J, Lipton MM, Thompson GE: Aspermatogenesis in the guinea pig induced by the testicular tissue and adjuvants. J Exp Med 1953;97:711-726.
54. Federal Drug Administration: FDA approves first injectable solution for dog sterilization. FDA Consumer 2003:3.
55. Neutersol: Neutersol information web site. Available at: www.neutersol.com/Study.htm Accessed: October 17, 2005.
56. Levy JK, Crawford PC, Appel LD, et al: Comparison of intratesticular injection of zinc gluconate versus surgical castration to sterilize male dogs. Am J Vet Res 2008;69:140-143.
57. Oliveira ECS, Moura MR, Silva FA, et al: Intratesticular injection of a zinc-based solution s a contraceptive for dogs. Theriogenology 2007:68:137-145
58. Naz RK, Talwar GP: Immunological sterilization of male dogs by BCG. Int J Androl 1981;4:111-128.
59. Fahim MS, Wang M, Sutcu MF, et al: Sterilization of dogs with intra-epididymal injection of zinc arginine. Contraception 1993;47:107-122.
60. Leonard A, Gerber GV, Leonard F: Mutagenicity, carcinogenicity and teratogenicity of zinc. Mutat Res 1987;168:343-8.
61. Fahim MS, Wang M, Stucu MF, et al: Sterilization of dogs with intra-epididymal injection of zinc arginine. Contraception 1993;47:107-22.
62. Bloomberg MS: Surgical neutering and non-surgical alternatives. J Am Vet Med Assoc 1996;208:517-519.
63. Immegart HI, Threlfall WR: Evaluation of intratesticular injection of glycerol for nonsurgical sterilization of dogs. Am J Vet Res 2000;61:544-9.
64. Tepsumethanon V, Wilde H, Hemachudha T: Intratesticular injection of a balanced zinc solution for permanent sterilization of dogs. J Med Assoc Thai 2005;88:686-689.
65. Esquivel-LaCroix C: Report on field use in 10,000 dogs in Mexico [abstract]. Proc Int Symp Non-Surg Contracept Pet Popul Control 2006. Available at: http://www.acc-d.org/2006%20Symposium%20Docs/5Esquivel_R.pdf. Accessed May 10, 2010.

66. Hawkins S, Costas G: Saving animals across borders; position on Neutersol [abstract]. Proc Int Symp Non-Surg Contracept Pet Popul Control, 2006. Available at http://www.acc-d.org/2006%20Symposium%20Docs/5Hawkins.pdf. Accessed: May 10, 2010.
67. Levy JK: Neutersol: what worked? What didn't? What's next? Session overview. Proc Int Symp Non-Surg Contracept Pet Popul Control 2006. Available at: http://www.acc-d.org/2006%20Symposium%20Docs/5Overview.pdf. Accessed: May 10, 2010.
68. Nolan RS: JAVMA News—Non-surgical alternatives explored as possible answers to dog and cat overpopulation. J Am Vet Med Assoc 2007;230:168-170.
69. Alliance for Contraception in Cats and Dogs: BN Research online survey with shelter veterinarians. Portland (OR): Alliance for Contraception in Cats and Dogs; 2008. p. 17.
70. Budke CM, Slater MR: Utilization of matrix population models to assess a 3-year single treatment nonsurgical contraception program versus surgical sterilization in feral cat populations. J Appl Anim Welf Sci 2009;12:277-292.

Semen collection, evaluation, and cooled shipment in the canine

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Abstract

The need for improvement in pregnancy rates and litter sizes has resulted in ever evolving theriogenology techniques. Over the past two decades, artificial insemination in the canine has progressed immensely. This reproductive technique has allowed increased genetic diversity and international breeding in multiple species. Another important advantage of artificial insemination is reduced disease transmission among breeding animals. Chilled transported semen is typically cooled to four to five degrees Celsius and can be conserved for up to 120 hours. A number of studies have evaluated various canine semen extenders and cooling techniques, with no single extender or technique found to be clearly superior. Methods for assessment of spermatozoa motility and fertility parameters include manual evaluation with a standard light microscope, various staining techniques, and computer assisted evaluation. These techniques vary in the degree of spermatozoa manipulation, technician experience required, and expense incurred.

Keywords: Canine, semen, semen extenders, shipping containers, CASA

Introduction

The initial description of the spermatozoa was provided by Leeuwenhoek in 1679.[1] Approximately 100 years later, the first artificial insemination in the canine species was performed by abbé Lazarro Spallanzani in Italy.[2] It was not until the early 1950's that artificial insemination in the dog using cooled extended semen resulted in a successful pregnancy.[3] The technique of artificial insemination with cryopreserved semen was not allowed by the American Kennel club until 1982.[4] Interestingly, the registration of offspring by extended cooled semen was not recognized until 1986.[4]

Pre-collection evaluation

Initial reproductive assessment of the canine patient should always begin with a thorough historical examination. The animal's age is important due to associated increased incidence of reproductive problems in the aged dog and transient infertility in the prepubertal dog.[5] Rijsselaere, et al. reported that age was significantly correlated with the percentage of normal spermatozoa but did not influence motility characteristics.[6] The reproductive history of the male, including the number of bitches bred, conception rate, litter size, and date of last collection or breeding, can assist with establishing the presence and duration of a reproductive problem. Serial breeding soundness examinations can also be useful for determination of changes in fertility and semen characteristics. If available, the breeding history of the dog's sire, dam, and littermates should also be evaluated.

It is also important to discuss the animal's previous medical history, attempting to identify any problems which could negatively influence fertility. Environmental, toxic, or physical insults can cause abnormal spermatozoal production for up to three months, potentially resulting in infertility during this time. Hereditable conditions such as hip dysplasia and degenerative myelopathy should also be investigated through assessment of the animal's pedigree and screening tests. *Brucella canis* testing and all routine vaccinations should be current in any breeding canine.

Physical examination of the male should be performed prior to collection. Thorough evaluation of the external genitalia and the prostate gland should be conducted. A rectal examination should always be performed to ensure the prostate gland is symmetrical in size and no pain is elicited upon digital palpation. Any abnormalities noted should be further investigated as they could suggest prostatic pathology such as benign prostatic hypertrophy, prostatic neoplasia, or prostatitis. Ultrasonography of the scrotum is beneficial for evaluation of the internal architecture of the testicles and epididymis. The testicular and epididymal texture and total scrotal width should be noted. Abnormalities such as adhesions, non-uniformity in size, fibrosis, and softness should also be recorded and investigated further.

Semen collection

It is important to note the date of the last ejaculation. If ejaculation has not occurred within the past ten days, secondary abnormalities associated with sperm cell aging and delayed spermatozoal transport, such as distal cytoplasmic droplets, could be observed in the first ejaculate. Frequently, a second collection following 30 minutes of sexual rest can result in a reduction in the number of these observed defects.

Prior to semen collection all equipment should be properly prepared. Microscope supplies, pipettes, and collection vessels should be warmed to 37 degrees Celsius to prevent excessive temperature fluctuations during semen handling. Rapid temperature changes can result in a loss of sperm motility and fertilizing ability. If lubricants are needed, small amounts of non-spermicidal lubricants or petroleum jelly are best due to the toxic effects of water soluble lubricants on spermatozoa. The extender should be prepared for use according to manufacturer recommendations and the collection vessel properly assembled prior to beginning the collection.

Digital manipulation is the most widely used method for semen collection in the dog and involves a collection vessel with or without a collection cone. The collection cone is usually composed of a sterilized plastic funnel-shaped sleeve that is attached to the collection vessel. The cone is occasionally not used because it has been associated with increased ejaculate contamination and increased difficulty of semen fractionation.

The canine artificial vagina is comprised of a rubber cone with a collection vessel attached to the funneled end. In one study, the rubber liner used in artificial vaginas resulted in deleterious effects on canine spermatozoa.[7] However, this study did provide data suggesting decreased toxic effects with repeated washing of the liner.[7] Electroejaculation is another collection method but is not commonly used in the canine patient due to the necessity of anesthesia for this procedure.

Urination should be prevented immediately prior to beginning ejaculation to avoid urine residue in the urethra. The animal should be restrained by a leash in a slip-free area that is void of distractions such as other animals and people. Although not necessary for males accustomed to manual stimulation, the presence of an estral bitch can assist with the collection process. Responsiveness to manipulation and a higher total number of spermatozoa in the ejaculate are commonly found when a teaser bitch is used.[8] If an estral bitch is unavailable, cotton tipped swabs which have been previously collected and frozen from a *Brucella canis* negative estral bitch can be used for olfactory stimulation.

The libido of the canine should be assessed as the collector begins to digitally manipulate the caudal aspect of the bulbus glandis. Pelvic thrusting normally begins quickly following manipulation. The collector should ensure that the preputial sheath is retracted fully caudal to the bulbus glandis prior to complete engorgement of the penis. If this is not possible, the collection process should be halted, the dog removed from the area until full detumescence is observed, and semen collection attempted again.

With retraction of the prepuce caudal to the bulbus glandis, the collector should slide the collection device over the penis, ensuring that there is no contact of the glans penis to the collection receptacle. A steady, firm pressure should be held caudal to the engorged bulbus glandis for the remainder of the collection. Allowing the male to step over the collector's arm or beginning collection with the penis directed caudally between the dog's rear legs enables a more normal ejaculation by stimulating a tie. If reluctance to ejaculate is encountered, the thumb of the hand holding the collection vial can be introduced into the fossa of the glans and pulsatile pressure applied to the urethral process against the wall of the vial.[9] To prevent injury and drying of the penile tissues, it is important to ensure full detumescence and full retraction of the penis into the preputial sheath following collection.

The ejaculate should be observed as it is deposited into the receptacle to determine the color and amount of each fraction. The first fraction, originating from the prostate gland, varies in volume, but is normally one to five milliliters. This fraction is clear to slightly opaque. The second fraction, also known as the sperm rich fraction, is between one to three milliliters and is cloudy-white in color. The third fraction, which is also prostatic in origin, is from two to 40 milliliters in volume and can be easily distinguished from the second fraction by its clearness. During discharge of the third fraction of the ejaculate, pulsations of the urethral sphincter can be observed by palpation of the penile shaft. It is important to note that in the canine the volume of the ejaculate does not correlate with the quality of the semen.

Depending on which extender is used or the purpose of the ejaculate, the three fractions may need to be separated during the collection process. It has been shown that the first and third ejaculate fractions are detrimental to spermatozoal motility and morphology during long term storage.[10] As a result, some practitioners prefer to only extend and ship the sperm rich, second fraction of the ejaculate. Fractionation of the ejaculate can be accomplished by forgoing usage of a collection cone and swapping pre-warmed collection vessels between the ejaculate fractions as they are collected. Separating the first and second fractions without partial mixing can be difficult, so these two fractions are often combined.

If there is uncertainty of ejaculation of the sperm rich fraction, alkaline phosphatase levels can be evaluated on the sample collected. This enzyme is produced in the epididymis and elevated levels in the sample validate presence of the second fraction. Seminal plasma alkaline phosphatase values suggestive of presence of the sperm rich fraction of the ejaculate are greater than 10,000 units per liter or a total measurement of greater than 30,000 units.[11]

Semen evaluation

A thorough semen evaluation includes assessment of the ejaculate volume, color, motility, concentration, and morphology. There are various procedures available to evaluate membrane integrity which can be associated with fertility. Ensuring collection equipment is clean, preventing heat and cold shock, and minimizing exposure to toxic insults will help ensure the findings of all examinations are reliable.

Color

Discoloration of the ejaculate is commonly associated with pathology. However, it is important to remember that color evaluation is a very subjective test. Semen with a yellowish hue may indicate urine contamination. An increased amount of white blood cells in the ejaculate can also result in semen with a slightly yellow appearance. A red to brown discoloration represents blood in the ejaculate which can be associated with prostatic disease or trauma prior to or during collection.

Motility

Motility, as an expression of viability and structural integrity, is one of the most important characteristics associated with the fertilizing ability of sperm.[12] The motility of the ejaculate should be observed as soon after collection as possible. The most commonly used method of motility analysis involves manual evaluation using a conventional light microscope at 100 to 200 times magnification. This is performed by placing a drop of semen on a microscopic slide and applying a warmed coverslip. Another commonly utilized method of motility assessment is the computer assisted semen analyzer (CASA). This equipment will be discussed in detail later in this manuscript.

To subjectively assess sperm motility, total and progressive motility should be evaluated by observing at least five fields within five minutes of placing the ejaculate on a warmed slide. Normal motility for canine patients is greater than 70 percent for both total and progressive motility. Progressive motility should be interpreted as spermatozoa that are moving forward in the field with speed. The percentage of progressively motile sperm has been positively correlated with the percentage of morphologically normal spermatozoa in dogs.[13] Mickelsen, et al. reported that canine pregnancy rates are significantly influenced by the total number of progressively motile or morphologically normal spermatozoa per ejaculate.[14]

Concentration

There is little value in attempting to use concentration alone as a correlation to semen quality.[15] The concentration of spermatozoa produced in each ejaculate varies greatly among dogs due to age, genetics, and environmental factors. Also, the total number of spermatozoa in an ejaculate is decreased with frequent semen collection.[8,16] Normal concentration of canine semen also depends on the weight of the dog and the breed, with a range of four to 400 million sperm per milliliter of ejaculate.[17] Mixed breeds tend to have higher sperm concentration.

The recommended insemination dose for fresh or chilled semen is 150 to 200 million progressively motile spermatozoa. With advances in artificial insemination procedures, the amount of spermatozoa inseminated can be decreased to as low as 10 million progressively motile fresh spermatozoa with acceptable pregnancy rates.[18] Due to the amplification of knowledge in artificial insemination in the canine, a single ejaculate can be used to inseminate multiple bitches with good pregnancy results.

Determination of spermatozoal concentration can be performed using optical density measurement, manual calculation using a hemacytometer, or CASA equipment. The hemacytometer technique is considered the gold standard. Each side of the hemacytometer should be evaluated to ensure that a concentration with less than ten percent variation is observed. Accurate calculation of concentration using the CASA system is a problem in every species. In the canine, the CASA system has been shown to overestimate concentration by 1.7 times.[19] Thus, the author does not recommend the use of the CASA system for determination of semen concentration in the dog.

Morphology

Morphology is a vital part of a complete semen evaluation. Oettlé reported that less than 60 percent normal morphology can adversely affect fertility.[1] Age, environment, and physical injury can result in a decrease in morphologically normal spermatozoa. The standard canine morphology guidelines allow for no less than 70 percent normal spermatozoa.

Although phase contrast microscopy is considered the gold standard, staining procedures with evaluation under light microscopy is the most commonly used method for morphology assessment. Stains that are customarily used for morphological examination are eosin-nigrosin and modified Wright's Giemsa. Eosin-nigrosin slides are prepared by mixing one drop of semen with one drop of stain and utilizing a "pusher" slide to spread the mixture

into a thin film. Spermatozoa that are stained with eosin-nigrosin appear white against a dark purple to black background. This stain is considered a vital stain due to the uptake of eosin by spermatozoa with non-intact or damaged membranes and these cells appear pink in color. Modified Wright's Giemsa stained slides are prepared by placing one drop of the semen sample on a slide which is then spread into a thin film. The sample slide is allowed to air dry and is then immersed into each of the three sequential stains for five minutes before rinsing with water. Wright's Giemsa stained spermatozoa are purple to pink in color on a white background. Acrosomal defects cannot be visualized using the modified Wright's Giemsa stain. A major benefit of using the modified Wright's Giemsa stain is that this staining procedure allows the differentiation of round cells, unlike when using eosin-nigrosin staining.

Examination for morphological characteristics is performed by observing the prepared slide under 1000 times magnification. Evaluation of at least 100 cells should be performed and the sperm should be divided into normal, primary abnormality, and secondary abnormality categories. Primary abnormalities are associated with spermatogenesis and include abnormalities such as proximal cytoplasmic droplets, bent midpieces, and defects involving the head of the spermatozoa. Secondary abnormalities occur as a result of faulty maturation or problems during epididymal transport. Such defects include, but are not limited to, distal cytoplasmic droplets, retroflexed tails, and detached heads.

Hypo-osmotic swelling test

Integrity of the plasmalemma has been associated with spermatozoal viability; thus, the hypo-osmotic swelling test is one method available to evaluate membrane integrity. An intact plasma membrane is important for sperm metabolism and changes in membrane properties that must occur for capacitation and the acrosome reaction to progress normally.[20]

The hypo-osmotic swelling test is a simple and inexpensive procedure. Various solutions including sucrose, fructose, and sodium citrate are utilized for this assay. Fructose is the most commonly used hypo-osmotic solution at a concentration ranging from 60 to 200 milliosmoles. One tenth of a milliliter of the semen sample is combined with one milliliter of the preferred hypo-osmotic solution. This mixture is incubated from one to 60 minutes at 37 degrees Celsius and is then evaluated under light microscopy or phase contrast microscopy. At least 100 sperm cells should be assessed for reaction to the hypo-osmotic media.

The spermatozoa respond to the hypo-osmotic solution by transporting water across the plasmalemma, resulting in swelling and curling if the membrane is intact during incubation. The sperm tail is particularly susceptible to changes in osmotic pressure; thus, most spermatozoa observed with a positive reaction to the hypo-osmotic swelling test reveal variations of tail curling. This affirmative reaction can range from curling of only the tip of the tail to a tightly coiled tail and suggests that the spermatozoal plasma membrane is intact or undamaged. Various studies have reported positive correlation between the hypo-osmotic swelling test response and sperm motility and viability in canines.[21,22] The hypo-osmotic swelling test has also been used effectively by Kumi-Diaka to observe subfertility in the canine patient.[20,23]

Computer assisted semen analysis

Computer assisted semen analysis was first described in the canine by Günzel-Apel, et al. in 1993.[24] With conventional microscopic analysis, variation in motility parameters of the same ejaculate can differ due to the subjective nature of this method. The usage of CASA systems provides an objective assessment of semen parameters by rapidly and accurately determining sperm motility and morphology. Although this equipment has great advantages, few practitioners have access to this method of analysis due to the cost and maintenance of the CASA system.

The CASA system produces sperm analysis results in the form of tables, graphs, and digital images of sperm cell tracks. This system can detect more subtle motion changes than conventional semen analysis. Computer assisted semen analysis also allows assessment of velocity parameters, linearity, straightness, beat cross frequency, and amplitude of lateral head displacement. As discussed previously, determination of semen concentration using CASA is not reliable.

The CASA system can evaluate semen parameters in any species, including humans, but the standardized system settings for each species should always be used. Several authors have reported high correlation between CASA results and conventional microscopic evaluation.[19,24] The accuracy of the resulting data depends greatly on the training and familiarity of the individual processing the samples. Another important factor in assessment of sperm motility parameters is the temperature of the sample at the time of evaluation. Iguer-Ouada and Verstegen revealed that motility parameters were decreased at 30 degrees Celsius, which corresponds with semen temperature

after ejaculation.[19] At 38 degrees Celsius, correlating with physiologic uterine temperature, motility parameters were more optimal.[19]

Dilution of the sample to approximately 50 million cells per milliliter is preferred to ensure adequate evaluation of individual spermatozoa and their motility and velocity components. Assessment of at least 200 cells reduces variability and misinterpretation of results. Semen should not be extended in whole milk or non-clarified egg yolk extenders when using CASA. These extenders contain particles approximately the same size as spermatozoal heads and can result in inaccurate motility assessment.[12] If these extenders must be used, DNA staining procedures can assist with differentiation between live sperm cells and inorganic particles.

Semen extenders

Canine artificial insemination with adequate numbers of freshly collected sperm can result in fertility rates equal to those obtainable with natural service.[25] Pinto, et al. reported that pregnancy rates and litter sizes were not different when dogs were bred with fresh or chilled extended semen that was stored for up to 48 hours.[4] The major objective of semen extension is conservation of spermatozoal motility and fertility during temperature changes and stress during shipment. Verstegen, et al. observed the ability to conserve chilled canine semen for up to 27 days, with addition of new semen extender at days 11, 21, and 27.[26] Spermatozoal motility was noted for up to 16 days with no exchange of semen extender.[26] In that study, motility was not significantly different than initial values up to day ten of evaluation and fertility was preserved for up to 11 days.[26]

Semen extenders provide energy, stabilize the pH and osmolarity, preserve cellular integrity during cooling of the sample, and can include antibiotics to assist with prevention of bacterial growth during transport or storage.[26] There is a variety of commercially available semen extenders, including egg yolk and milk based solutions. The addition of egg yolk to extenders has a protective effect by preserving motility parameters and is commonly added to extenders for semen cryopreservation.[27] The major effect of glucose and fructose in semen extenders is to support spermatozoal motility and movement patterns during storage.[28] Antibiotics are especially important when using an egg yolk based extender due to the increased risk of bacterial growth. The two most frequently used antibiotics in semen extenders are amikacin and gentamicin which provide protection against gram negative bacterial growth. In 1992, Bjurström and Linde-Forsberg reported that the most common organisms cultured from preputial samples were *Pasteurella multocida*, β-hemolytic streptococci, and *Escherichia coli*.[29] They also stated that the most commonly cultured bacteria from semen were *Pasteurella multocida* and β-hemolytic streptococci.[29]

Extender preparation varies due to manufacturer and extender type. Some commercially available extenders should be warmed to room temperature prior to combining with the ejaculate, versus 37 degrees Celsius as recommended by other semen extender manufacturers. Some manufacturers recommend extending only the second ejaculate fraction to achieve maximal fertility and motility throughout storage or shipment. The need for equilibration is another component of the extension process that varies depending on the semen extender type and manufacturer. Homemade extenders can also be used but require increased preparation time and there is variable consistency between preparations. Thus, commercially available extenders with less strict instructions on preparation, collection, and equilibration could be considered more efficient and user friendly.

Most commercially available extenders suggest a dilution ratio of at least one part semen to four parts extender for maximal motility and fertility during cooled shipment or storage. Depending on the concentration of the ejaculate, centrifugation and re-extension of the sample might be necessary prior to packaging. This should be decided on an individual case basis as the need arises. It is important to ensure the insemination volume is between five and ten milliliters for vaginal artificial insemination and approximately two milliliters for intrauterine insemination. This volume can be adjusted in relation to breed and size of the bitch.

It is beneficial to evaluate the semen of a new patient in different extenders and shipment containers to determine which technique or product is best for that particular patient or the situation of insemination or shipment. This is especially important in animals with known subfertility or infertility.

Semen shipment

Semen transport for insemination purposes has become very common over the past two decades due to convenient overnight delivery services. Artificial insemination allows national and international breeding with chilled or cryopreserved shipped semen. Studies have previously found pregnancy rates of approximately 80 percent with natural matings, good quality fresh, chilled extended semen, or combinations of the above.[25,30,31,32]

Extended semen should be placed in plastic syringes or other plastic or glass vessels with secure lids for storage. A study performed by England and Allen revealed toxic effects of disposable plastic syringe components, particularly rubber plungers.[7] Due to this finding, washing syringes or using syringes without rubber components are common practices when storing or transporting semen.

A study by Michael, et al. revealed that during shipment, semen quality always deteriorated in a gradual, constant, and expected manner.[33] Chilling the sample during storage lowers the metabolic rate, resulting in increased spermatozoal longevity. Sperm quality and longevity following storage can be affected by the temperature to which the semen is cooled. Semen being transported for insemination purposes should be chilled to four to five degrees Celsius, as determined by various studies throughout the last decade.[23,25,27,34-38] Canine semen stored at four degrees Celsius has a significantly longer life span than semen stored at 22 degrees Celsius.[2] Semen parameters are also maintained for longer periods when stored at 22 degrees Celsius than at 37 degrees Celsius.[2]

The amount of time the sample is stored prior to insemination is also an important factor in semen quality following storage. Although a variety of investigators have found viability of spermatozoa for approximately four to five days,[35,39] semen can retain good quality for at least 24 hours under ideal packaging and shipment conditions.[2,4,9,25,35,40,41] Due to this finding, most shipped semen is used within the first 48 hours of storage.

The maintenance of semen quality throughout shipment or storage is also affected by the shipment container employed. There are various commercially available semen transport containers, including the vacuum flask (Thermos®, Thermos LLC, Rolling Meadows, IL), extruded polystyrene foam (Styrofoam®, Dow Chemical, Midland, MI) box, and plastic box, such as the Equitainer® (Hamilton Research, Inc, South Hamilton, MA). Although reuse is possible with proper care, both the vacuum flask and extruded polystyrene foam box are considered single use shipment containers. Shipment using the vacuum flask is inexpensive due to the low purchase cost and the small size of the container. Vacuum flasks have no defined cooling rates or temperature holding ability because of variability in models and sample preparation.[40] The extruded polystyrene foam box is fairly inexpensive and can be purchased from various companies. The Equitainer® is the only semen shipment container available that is marketed for reuse. This container is associated with increased initial purchase expense and shipping costs due to the increased weight.

A single shipment container has not been proven as the gold standard for maintenance of semen quality following storage. A study performed by Lopes, et al. demonstrated advantages of the Equitainer® over the extruded polystyrene foam box and the vacuum flask when transporting chilled canine semen for more than 48 hours.[40] A higher percentage of progressive motility was observed when using the Equitainer® in this study.[40] Another report evaluating the Equitainer® and a low cost extruded polystyrene foam box using equine semen revealed that the low cost system was satisfactory for cooling and preserving equine semen for up to 48 hours of storage.[42] In this study, the low cost container did reach temperatures of less than four degrees Celsius, which was thought to be detrimental.[42] No impairment on semen quality was observed.[42] The Equine Express II™ box (Nasco, Ft. Atkinson, WI or Modesto, CA and Exodus Breeders Corporation, York, PA) is a commercially used storage container for cooled semen shipment in the stallion. Katila, et al. compared the usage of the Equine Express II™ box, the Equitainer®, and the ExpectaFoal™ box for cooled semen shipment in the stallion.[43] This study revealed similar motility parameters in each transport container following storage of semen for 24 hours.[43]

Storage temperature is a vital component of cooled semen shipment quality. The most commonly used temperature for storage of semen is four to five degrees Celsius. A study performed by Brinsko, et al. demonstrated that the Equitainer®, Bio-Flite™ (Bio Flite, Anaheim, CA), Lane STS™ (Lane Manufacturing, Denver, CO), and Equine Express II™ shipment containers retained a temperature greater than six degrees Celsius at an environmental temperature of 22 degrees Celsius or 37 degrees Celsius.[44] No adverse affects on spermatozoal motility were reported.[44] Thus, containers maintaining temperatures greater than four to five degrees Celsius may not be detrimental to spermatozoal quality after storage or shipment.

Environmental storage temperature is also an important factor of semen quality following storage. The recent report from Brinsko, et al. suggests that the majority of available shipment containers adequately maintain appropriate storage temperatures under typical ambient conditions.[44] An evaluation of the Equitainer® and extruded polystyrene foam box was performed by Malmgren in 1998 to determine the effectiveness of the two transport containers at maintaining semen quality during 24 hours of storage at varying ambient temperatures.[41] The author reported that the Equitainer® maintained better semen quality at 37 degrees Celsius than at 20 degrees Celsius ambient temperature and for a longer time period.[41]

Conclusion

Semen transport is a very common procedure in canine theriogenology. There are various commercially available and homemade semen extenders that allow preservation of semen during transport or storage. Semen shipment containers available for chilled transport include the standard vacuum flask, various extruded polystyrene foam boxes, and the Equitainer®. Although previous studies have found one extender or shipment container to be superior, without uniformity in the resulting data, an ultimate semen extender and shipment container have not been defined.

Although the best evaluation of semen extenders and shipping techniques is conception rates after well-timed matings, the prolonged interestral interval of the canine patient prevents substantial fertility research in this species. The various components of standard semen evaluation can assist in determining the fertility potential of breeding canines. Although computer assisted semen analysis is an expensive procedure and requires a skilled operator, utilization of this objective assessment of motility and morphology is becoming a mainstay for semen evaluation in theriogenology. This system coupled with inexpensive and simple microscopic evaluation can result in a thorough evaluation of semen quality in the canine patient.

References

1. Oettlé EE: Sperm morphology and fertility in the dog. J Reprod Fertil 1993;47(Suppl):257-260.
2. Linde-Forsberg C: Artificial insemination with fresh, chilled extended, and frozen-thawed semen in the dog. Semin Vet Med Surg Small Anim 1995;10:48-58.
3. Harrop AE: Artificial insemination of a bitch with preserved semen. Br Vet J 1954;110:424-425.
4. Pinto CRF, Paccamonti DL, Eilts BE: Fertility in bitches artificially inseminated with extended, chilled semen. Theriogenology 1999;52:609-616.
5. Purswell BJ, Althouse GC, Root Kustritz MV: Guidelines for using the canine breeding soundness evaluation form. Clin Therio 2010;2:51-59.
6. Rijsselaere T, Maes D, Hoflack G, et al: Effect of body weight, age and breeding history on canine sperm quality parameters measured by the Hamilton-Thorne analyser. Reprod Domest Anim 2007;42:143-148.
7. England GCW, Allen WE: Factors affecting the viability of canine spermatozoa. I. Potential influences during processing for artificial insemination. Theriogenology 1992;37:363-371.
8. Boucher JH, Foote RH, Kirk RW: The evaluation of semen quality in the dog and the effects of frequency of ejaculation upon semen quality, libido, and depletion of sperm reserves. Cornell Vet 1958;48:67-86.
9. Linde-Forsberg C: Achieving canine pregnancy by using frozen or chilled extended semen. Vet Clin North Am Small Anim Pract 1991;21:467-485.
10. England GCW, Allen WE: Factors affecting the viability of canine spermatozoa. II. Effects of seminal plasma and blood. Theriogenology 1992;37:373-381.
11. Kutzler MA, Solter PF, Hoffman WE, et al: Characterization and localization of alkaline phosphatase in canine seminal plasma and gonadal tissues. Theriogenology 2003;60:299-306.
12. Verstegen J, Iguer-Ouada M, Onclin K: Computer assisted semen analyzers in andrology research and veterinary practice. Theriogenology 2002;57:149-179.
13. Ellington J, Scarlett J, Meyers-Wallen V, et al: Computer-assisted sperm analysis of canine spermatozoa motility measurements. Theriogenology 1993;40:725-733.
14. Mickelsen WD, Memon MA, Anderson PB, et al: The relationship of semen quality to pregnancy rate and litter size following artificial insemination in the bitch. Theriogenology 1993;39:553-560.
15. Root Kustritz MV: The value of canine semen evaluation for practitioners. Theriogenology 2007;68:329-337.
16. England GCW: Semen quality in dogs and the influence of a short-interval second ejaculation. Theriogenology 1999;52:981-986.
17. Johnston SD, Root Kustritz MV, Olson PNS: Canine and feline theriogenology. Philadelphia: Saunders; 2001. p. 293.
18. Tsutsui T, Shimizu T, Ohara N, et al: Relationship between the number of sperms and the rate of implantation in bitches inseminated into unilateral uterine horn. Jpn J Vet Sci 1989;51:257-263.
19. Iguer-Ouada M, Verstegen JP: Evaluation of the "Hamilton Thorn computer-based automated system" for dog semen analysis. Theriogenology 2001;55:733-749.
20. Kumi-Diaka J: Subjecting canine semen to the hypo-osmotic test. Theriogenology 1993;39:1279-1289.
21. Pinto CRF, Kozink DM: Simplified hypoosmotic swelling test (HOST) of fresh and frozen-thawed canine spermatozoa. Anim Reprod Sci 2008;104:450-455.
22. Rodríguez-Gil JE, Montserrat A, Rigau T: Effects of hypoosmotic incubation on acrosome and tail structure on canine spermatozoa. Theriogenology 1994;42:815-829.
23. Kumi-Diaka J, Badtram G: Effect of storage on sperm membrane integrity and other functional characteristics of canine spermatozoa: in vitro bioassay for canine semen. Theriogenology 1994;41:1355-1366.
24. Günzel-Apel AR, Günther C, Terhaer P, et al: Computer-assisted analysis of motility, velocity and linearity of dog spermatozoa. J Reprod Fertil 1993;47(Suppl):271-278.
25. Gill HP, Kaufman CF, Foote RH, et al: Artificial insemination of beagle bitches with freshly collected, liquid-stored, and frozen-stored semen. Am J Vet Res 1970;31:1807-1813.
26. Verstegen JP, Onclin K, Iguer-Ouada M: Long-term motility and fertility conservation of chilled canine semen using egg yolk added Tris-glucose extender: in vitro and in vivo studies. Theriogenology 2005;64:720-733.
27. Iguer-Ouada M, Verstegen JP: Long-term preservation of chilled canine semen: effect of commercial and laboratory prepared extenders. Theriogenology 2001;55:671-684.
28. Ponglowhapan S, Essén-Gustavsson B, Linde Forsberg C: Influence of glucose and fructose in the extender during long-term storage of chilled canine semen. Theriogenology 2004;62:1498-1517.
29. Bjurström L, Linde-Forsberg C: Long-term study of aerobic bacteria of the genital tract in stud dogs. Am J Vet Res 1992;53:670-673.
30. England GCW, Allen WE: Seminal characteristics and fertility in dogs. Vet Rec 1989;125:399.
31. Farstad W: Bitch fertility after natural mating and after artificial insemination with fresh or frozen semen. J Small Anim Pract 1984;25:561-565.
32. Linde-Forsberg C, Forsberg M: Results of 527 controlled artificial inseminations in dogs. J Reprod Fertil 1993;47(Suppl):313-323.
33. Michael AJ, Alexopoulos C, Pontiki EA, et al: Effect of antioxidant supplementation in semen extenders on semen quality and reactive oxygen species of chilled canine spermatozoa. Anim Reprod Sci 2009;112:119-135.
34. Bouchard GF, Morris JK, Sikes JD, et al: Effect of storage temperature, cooling rates and two different semen extenders on canine spermatozoa motility. Theriogenology 1990;34:147-157.
35. Province CA, Amann RP, Pickett BW, et al: Extenders for preservation of canine and equine spermatozoa at 5°C. Theriogenology 1984;22:409-415.

36. Aurich C: Recent advances in cooled-semen technology. Anim Reprod Sci 2008;107:268-275.
37. Moran DM, Jasko DJ, Squires EL, et al: Determination of temperature and cooling rate which induce cold shock in stallion spermatozoa. Theriogenology 1992;38:999-1012.
38. Varner DD, Blanchard TL, Meyers PJ, et al: Fertilizing capacity of equine spermatozoa stored for 24 hours at 5 or 20°C. Theriogenology 1989;32:515-525.
39. Rota A, Ström B, Linde-Forsberg C: Effects of seminal plasma and three extenders on canine semen stored at 4°C. Theriogenology 1995;44:885-900.
40. Lopes G, Simões A, Ferreira P, et al: Differences in preservation of canine chilled semen using different transport containers. Anim Reprod Sci 2009;112:158-163.
41. Malmgren L: Effectiveness of two systems for transporting equine semen. Theriogenology 1998;50:833-839.
42. Nunes DB, Zorzatto JR, Costa e Silva EV, et al: Efficiency of short-term storage of equine semen in a simple-design cooling system. Anim Reprod Sci 2008;104:434-439.
43. Katila T, Combes GB, Varner DD, et al: Comparison of three containers used for the transport of cooled stallion semen. Theriogenology 1997;48:1085-1092.
44. Brinsko SP, Rowan KR, Varner DD, et al: Effects of transport container and ambient storage temperature on motion characteristics of equine spermatozoa. Theriogenology 2000;53:1641-1655.

Feline reproduction FAQs

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Introduction

Many veterinarians graduate with no formal education in feline reproduction; cats were not identified as one of the "major" species in which theriogenology training was required at most schools surveyed.[1] In a study in which practitioners across the United States were asked to rank which procedures in theriogenology they performed most commonly by species, dystocia management and treatment of reproductive tract disease were those listed most commonly for the cat.[2] This mirrors a survey of veterinarians on the Society for Theriogenology small animal list-serve, who rated dystocia management, diagnosis and treatment of pyometra, broad management of infertility, and ovarian remnant syndrome as the most common disorders presented in feline reproduction in their practices. This is a review of three common disorders seen in feline reproductive practice.

Keywords: Feline, dystocia, pyometra, ovarian remnant syndrome, reproductive disease

Dystocia

Dystocia, abnormal queening, is reported to occur in 3 to 6% of cat parturitions.[3,4] Veterinary students report great fear around dystocia and practitioners mimic this concern, perhaps because of the possibility of impacting several lives. There are several excellent recent reviews of dystocia.[5,6] The reader is referred to these for greater detail.

Dystocia can be maternal or fetal in origin. The most common reported maternal cause is uterine inertia, lack of synchronous uterine contractions. The most common reported fetal cause is malpresentation, due to oversize or abnormal orientation of the limbs or head relative to the spine.[7] Brachycephalic and dolicocephalic breeds are at increased risk.[4,7] Dystocia is present if gestation is prolonged, labor is not progressing, the queen appears systemically ill, or the fetuses are in distress.

Normal gestation length is queens averages 66.9 days and varies from 62 to 71 days.[8] Despite induction of ovulation and a commonly restricted number of days of breeding, gestation length is truly this variable and cannot be estimated based on other factors such as litter size. Therefore, gestation is not automatically considered prolonged unless the queen is at least 71 days past the last known breeding.

Normal parturition length in cats is prolonged compared to dogs. Mean parturition length is 16.1 hours with a range of four to 42 hours.[8] In the author's colony, one cat queened four kittens over three calendar days; one of them was stillborn but it was the third one born, not the final one.[8] In general, the first kitten should be passed within four hours of onset of active labor and subsequent kittens passed at least every two hours. Because queens apparently may inhibit labor voluntarily due to stress, the queen should undergo a complete physical examination and the kittens should be assessed for viability by verification of heart rate greater than 170 to 200 bpm as part of any decision whether to intervene.

Medical therapy is reported to be effective in cats only 29.9% of the time that it is attempted.[7] Whether this is due to medical treatment being used when surgical treatment is called for or to variable responses of queens to medical therapy with oxytocin compared to other species is not clear. Radiography is recommended to best predict whether fetuses are of a size suitable for vaginal delivery.

Medical therapy includes administration of oxytocin (0.1 to 0.25 IU SQ or IM) and calcium gluconate (10% solution, 0.5 to 1.0 ml SQ or IM).[5] If the cat does not respond after one or two injections at 20 to 30 minute intervals, surgical treatment by cesarean section is recommended. In one survey of 1056 births, 8% were resolved by cesarean section.[9]

Pyometra

Pyometra is uterine enlargement due to accumulation of purulent fluid. This condition frequently is associated with cystic changes in the endometrium (cystic endometrial hyperplasia [CEH]). The pathogenesis in cats is not completely clear. While several surveys have demonstrated increased incidence in cats greater than five years of age, pyometra has been documented in many younger cats and has a reported overall incidence of about 0.4%.[10-12] Dow described four stages of CEH-pyometra, with increasing degrees of inflammation and atrophy overlying CEH; mean age of cats representative of all four groups was the same, suggesting this is not a progressive disease.[13] Cystic endometrial hyperplasia can be induced experimentally by treatment with progestogens and is considered a side-effect of progestogen therapy but affected cats do not always have high serum progesterone concentrations nor is luteal tissue always present.[10,14-16] It may be that queens, like bitches, may develop two forms

of endometrial hyperplasia, one a chronic cystic form and the other a transient proliferative form.[17] One study suggested that cats may be less likely to present with pyometra during seasonal anestrus in November through February.[13]

Because cats are induced ovulators, one perception was that queens would be less likely to develop pyometra if they were never induced to ovulate. However, many young queens who were known not to have been induced to ovulate have presented with pyometra. It has been reported that up to 22% of queens presenting with pyometra have no recent history of estrus.[18] It has been demonstrated that queens will occasionally demonstrate a rise in serum progesterone or presence of luteal tissue in the absence of a known stimulus for ovulation.[16,19] This suggests that pyometra must be on the rule-out list for any intact queen with signs of systemic disease.

The most common clinical signs reported in queens with pyometra are lethargy, anorexia, and vulvar discharge and abdominal distension, depending on cervical patency. Diagnostic findings on physical examination include fever, dehydration, a palpably enlarged uterus, and vulvar discharge if the cervix is open.[15,20] On complete blood count, non-regenerative anemia is more common than in dogs, with that anemia more severe than that seen in dogs.[11] Leukocytosis with a left shift is usually present and length of hospital stay is positively associated with white blood cell number and percentage bands.[15,21] Physical findings and changes on labwork may be a manifestation of systemic inflammatory response syndrome, associated with sepsis.[21,22] Uterine enlargement may be evident on radiographs and is easily identified on ultrasound.[18,20] *E. coli* is the most common bacterial organism isolated.[16]

Ovariohysterectomy is the best treatment. Medical therapy may be attempted in young, valuable queens with open-cervix pyometra. The most common therapy used in the United States is prostaglandin F2alpha (0.1 to 0.25 mg/kg SQ BID until uterus nears normal size or all free uterine fluid is cleared) with concurrent antibiotic therapy; antibiotic choice should be based on culture and sensitivity testing and should continue until vulvar discharge has not been seen for one week.[18] Agleprisone (Alizine™, Virbac Laboratories, Carros, France), a progesterone-receptor blocker, is reported as a successful therapy in other countries when used at a dose of 10 mg/kg SQ on days 1, 2, 7 and, if necessary, 14.[15] This drug is not currently available in the United States.

Ovarian remnant syndrome

Ovarian remnant syndrome (ORS) is the presence of signs of estrus in a queen who had previously undergone ovariohysterectomy (OHE). Signs of estrus may appear anytime from 14 days to nine years from the date of surgery.[23,24] Once these signs appear, the cat usually shows normal periodicity and seasonality of estrus signs including lordosis, vocalization, and roaming behavior.

The cause of ORS in cats is not well understood. Many times it is obvious surgeon error with whole ovaries or pieces of uterus identified.[25,26] It has been demonstrated that ovarian tissue dropped into the abdominal cavity can revascularize and become functional, making it possible that any ovarian tissue caught in a clamp could undergo the same reaction.[27,28] However, it has been demonstrated that incidence of ORS is not associated with reason for OHE (elective versus as treatment of uterine or ovarian disease) or with experience level of the veterinarian performing the surgery.[24] It has been hypothesized that some cats may have an extra piece of ovarian tissue deep to the main ovary that is not removed during routine OHE and becomes functional after removal of the main ovary.

Cats are polyestrus and induced ovulators and these facts guide diagnosis. The cat is best examined when the owner perceives her to be in heat. If an active follicle is present, the vaginal epithelial cells will be cornified. Cornified vaginal epithelial cells are larger than non-cornified cells and misshapen but do not form the large clumped sheets seen in bitches.[29] Identification of cornified cells is a better indicator of elevated estrogen concentrations than is measurement of serum estrogen. If a follicle is present, evidenced by cornified vaginal cytology, exploratory surgery can be performed at that time to identify and remove the ovarian remnant with the follicles on it, or luteinization of that tissue can be induced with gonadotropin releasing hormone (GnRH; 25 mcg IM) or human chorionic gonadotropin (hCG; 500 IU IM).[25,30] Blood should be drawn two to three weeks later and progesterone assayed. Serum progesterone concentration greater than 2 ng/ml is indicative of luteal tissue. This is definitive for diagnosis of ORS because only ovarian tissue can make estrogen and be induced to make progesterone by administration of GnRH. Ultrasound was reported to be an effective diagnostic tool in nine of 12 cases of ORS in dogs and cats in one study; operator skills and equipment are limiting factors for this technique in practice.[26]

Surgery is strongly recommended. Even though the cat is unlikely to be able to be able to become pregnant because of previous hysterectomy, persistent ovarian function will predispose the cat the mammary neoplasia. There are no estrus suppressing drugs that are safe for long-term use in cats. Surgery should be performed when the remnant tissue is more obvious, either when follicles or luteal tissue is present. The author prefers to perform

surgery when luteal tissue is present because the disorder has been diagnosed definitively as previously described and because luteal tissue persists for an average of 40 days after GnRH administration.

Ovarian remnants may be found at one or both ovarian pedicles and may occasionally be found at other sites, including the linea alba, dorsal body wall, and kidney capsule.[26,*,†] If no obvious ovarian tissue is found, the scar tissue at both pedicles should be removed. All excised tissue should be submitted for histopathology; teratoma and granulosa cell tumors have been identified in ovarian remnants.[23,31]

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* Gartley C: personal communication, July 25, 2000.
†Thomas P: personal communication, July 25, 2000.

References

1. Root Kustritz MV, Tibary A, Chenoweth PJ: Availability of theriogenology training at North American and Caribbean veterinary colleges. J Vet Med Educ 2006;33:140-144.
2. Humphreys J: Dystocia in cats. Vet Rec 1974;95:353.
3. Root Kustritz MV, Chenoweth P, Tibary A: Effect of training in theriogenology as determined by a survey of veterinarians. J Am Vet Med Assoc 2006;229:514-521.
4. Gunn-Moore DA, Thrusfield MV: Feline dystocia: prevalence, and association with cranial conformation and breed. Vet Rec 1995;136:350-353.
5. Pretzer SD: Medical management of canine and feline dystocia. Theriogenology 2008;70:332-336.
6. Traas AM: Surgical management of canine and feline dystocia. Theriogenology 2008;70:337-342.
7. Ekstrand C, Linde-Forsberg C: Dystocia in the cat: a retrospective study of 155 cases. J Sm Anim Pract 1994;35:459-464.
8. Root MV, Johnston SD, Olson PN: Estrous length, pregnancy rate, gestation and parturition lengths, litter size, and juvenile mortality in the domestic cat. J Am Anim Hosp Assoc 1995;31:429-433.
9. Root Kustritz MV: Clinical canine and feline reproduction: evidence-based answers. Ames (IA):Wiley-Blackwell; 2010. p. 189-190.
10. Potter K, Hancock DH, Gallina AM: Clinical and pathologic features of endometrial hyperplasia, pyometra, and endometritis in cats: 79 cases (1980-1985). J Am Vet Med Assoc 1991;198:1427-1431.
11. Wheaton LG, Johnson AL, Parker AJ, et al: Results and complications of surgical treatment of pyometra: a review of 80 cases. J Am Anim Hosp Assoc 1989;25:563-568.
12. Scott KC, Levy JK, Crawford PC: Characteristics of free-roaming cats evaluated in a trap-neuter-return program. J Am Vet Med Assoc 2002;221:1136-1138.
13. Dow C: The cystic hyperplasia-pyometra complex in the cat. Vet Rec 1962;74:141-147.
14. Keskin A, Yilmazbas G, Yilmaz R, et al: Pathological abnormalities after long-term administration of medroxyprogesterone acetate in a queen. J Feline Med Surg 2009;11:518-521.
15. Nak D, Nak Y, Tuna B: Follow-up examinations after medical treatment of pyometra in cats with the progesterone-antagonist aglepristone. J Feline Med Surg 2009;11:499-502.
16. Lawler DF, Evans RH, Reimers TJ, et al: Histopathologic features, environmental factors, and serum estrogen, progesterone, and prolactin values associated with ovarian phase and inflammatory uterine disease in cats. Am J Vet Res 1991;52:1747-1753.
17. Schlafer DH, Gifford AT: Cystic endometrial hyperplasia, pseudo-placentational endometrial hyperplasia, and other cystic conditions of the canine and feline uterus. Theriogenology 2008;70:349-358.
18. Root Kustritz MV: Clinical canine and feline reproduction: evidence-based answers. Ames (IA): Wiley-Blackwell; 2010. p. 245-247.
19. Lawler DF, Johnston SD, Hegstad RL, et al: Ovulation without cervical stimulation in domestic cats. J Reprod Fertil 1993;Suppl 47:57-61.
20. Kenney KJ, Matthiesen DT, Brown NO, et al: Pyometra in cats: 183 cases (1979-1984). J Am Vet Med Assoc 1987;191:1130-1132.
21. Hagman R, Karlstam E, Persson S, et al: Plasma PGF2alpha metabolite levels in cats with uterine disease. Theriogenology 2009;72:1180-1187.
22. Brady CA, Otto CM, vanWinkle TJ, et al: Severe sepsis in cats: 29 cases (1986-1998). J Am Vet Med Assoc 2000;217:531-535.
23. Wallace MS: The ovarian remnant syndrome in the bitch and queen. Vet Clin North Am Small Anim Pract 1991;21:501-507.
24. Miller DM: Ovarian remnant syndrome in dogs and cats: 46 cases (1988-1992). J Vet Diagn Invest 1995;7:572-574.
25. England GCW: Confirmation of ovarian remnant syndrome in the queen using hCG administration. Vet Rec 1997;141:309-310.
26. Ball RL, Birchard SJ, May LR: Ovarian remnant syndrome in dogs and cats: 21 cases (2000-2007). J Am Vet Med Assoc 2010;236:548-553.
27. Shemwell RE, Weed JC: Ovarian remnant syndrome. Obstet Gyne 1970;36:299-303.
28. DeNardo GA, Becker K, Brown NO, et al: Ovarian remnant syndrome: revascularization of free-floating ovarian tissue in the feline abdominal cavity. J Am Anim Hosp Assoc 2001;37:290-296.
29. Root Kustritz MV: Clinical canine and feline reproduction: evidence-based answers. Ames (IA): Wiley-Blackwell;2010. p. 189-190.
30. Root Kustritz MV: Clinical canine and feline reproduction: evidence-based answers. Ames (IA):Wiley-Blackwell;2010. p. 255-256.
31. Phil Thomas, personal communication, July 25, 2000.
32. Root Kustritz MV, Rudolph KD: Theriogenology question of the month: teratoma on an ovarian remnant in a cat. J Am Vet Med Assoc 2001;219:1065-1066.

Semen collection, evaluation, and cryopreservation in the domestic feline

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Abstract

Semen collection, cryopreservation, and subsequent use in the domestic cat has its own unique challenges, but is important for the improvement of advanced reproductive techniques. The number of publications on this topic has increased dramatically over the past ten years. The domestic cat serves as an important model for several human diseases, as well as a model for the preservation of the rare and endangered feline species. Techniques for the preservation of spermatozoa in the domestic cat are vital to preserve valuable genetics in pure breed catteries and valuable research models. Semen collection is most often performed by use of an artificial vagina, or by electroejaculation under general anesthesia. The intent of this review is to describe a detailed protocol for the successful collection, cryopreservation, and use of feline semen.

Keywords: Feline, cat, cryopreservation, sperm collection, electroejaculation

Introduction

Interest in domestic cat semen collection, evaluation, and cryopreservation has increased in the past ten years. Because the domestic cat can serve as a model for reproduction of exotic and endangered feline species, and as an animal model for human disease, interest in assisted reproduction has increased. Semen collection and evaluation in the cat present a unique set of challenges due to the small volume and comparatively low sperm numbers present in each ejaculate. In the domestic cat, semen collection, evaluation, and cryopreservation are used extensively in catteries and feline research colonies to preserve important or valuable genetic materials.

Semen collection

The two common methods of semen collection in the tom cat are an artificial vagina (AV) and electroejaculation. The AV is most commonly constructed using an Eppendorf tube (Eppendorf North America, Hauppauge, NY) and a rubber pipette bulb. The male is allowed to mount a queen, and the AV is held in place to facilitate and collect the ejaculate. If the male has been adequately trained to the AV, or a queen in estrus is not available, the male may be allowed to mount a gloved arm. The advantages of using an AV are that collection can be performed readily in the unanesthetized tom and a complete ejaculate is obtained. Disadvantages are the requirement for training and the frequent necessity of a teaser queen. Often, two to three weeks of conditioning and training are required before a tom is consistently producing ejaculates, and training may not be successful in all toms. Collection using an AV is an excellent method for situations where a single male or group of toms are collected on a regular basis, such as in a cattery or research colony, but is impractical for a single evaluation in a clinical setting using an untrained tom.

Electroejaculation is the most common method of obtaining an ejaculate from a tom which is not trained to an AV. The procedure requires general anesthesia. This author's preferred anesthesia protocol includes dexmedetomidine (30-40 μg/kg) and ketamine (3-5 mg/kg) injected intramuscularly, followed by intubation and supplemental oxygen. Inhalant anesthesia (isofluorane) can be added if necessary, but the short procedure time generally does not require it. On occasion, electroejaculation using inhalation anesthetics may result in urination and contamination of semen samples. Zambelli, et al. compared the quality of ejaculates collected by electroejaculation using medetomidine alone or ketamine alone. These researchers found that the use of an α_2 agonist (medetomidine) produced higher numbers of spermatozoa in the ejaculate than using ketamine alone, and did not increase the incidence of retroejaculation.[1] However, these researchers did not evaluate the use of these medications in combination. To prevent the perception of discomfort during the procedure, it is recommended that an anesthetic, such as ketamine, be added to balance the sedative and analgesic effects of dexmedetomidine (personal communication, Johnson 2010). Each tom should be monitored appropriately while under general anesthesia to minimize anesthetic complications.

Procedure for electroejaculation:

- Electroejaculation is performed using a rectal probe one cm in diameter and 12-13 cm long (Figure 1). Appropriate electroejaculators are available commercially (P-T Electronics, Boring, OR; Figure 2).
- Lubricate the rectal probe with non-spermicidal lubricant and insert the probe gently into the rectum approximately 5-7 cm. The electrodes should be oriented ventrally.

- If feces in the rectum prevent the placement of the probe, a lubricated gloved finger may be used to evacuate the rectum but is not always necessary.
- Manually extend the penis and clean with gauze moistened with saline (no alcohol or soap). Dry the penis with clean or sterile dry gauze.
- Place a sterile vial (Eppendorf) over the penis. Alternatively, one could use a sterile 5 mL sample collection vial (Nalgene, Thermo Fisher Scientific, Rochester, NY).
- Turn on the ejaculator. Make sure the rheostat dial is set to zero prior to activating the power switch.
- Rotate the rheostat to provide a series of electrical stimuli by turning the dial to the desired voltage for 2-3 seconds, then abruptly back to zero for 2-3 seconds. The stimuli should be administered in the following order:
    - Set one: 10 times with 2 volts, 10 times with 3 volts, 10 times with 4 volts, rest 3-5 min.
    - Set two: 10 times with 3 volts, 10 times with 4 volts, 10 times with 5 volts, rest 3-5 min
    - Set 3: 10 times with 4 volts, 10 times with 5 volts, 10 times with 5 volts (or 6 if needed, depending on previous response).
- The sample obtained between each electroejaculation set should be evaluated for the presence of sperm. This is usually readily evident, as an ejaculate containing spermatozoa will be cloudy. A new, sterile tube should be used between each set after collection to prevent contamination or loss of the sample.

Figure 1: A rectal probe designed for use in the domestic feline measures 1 cm in diameter and 12-13 cm long.

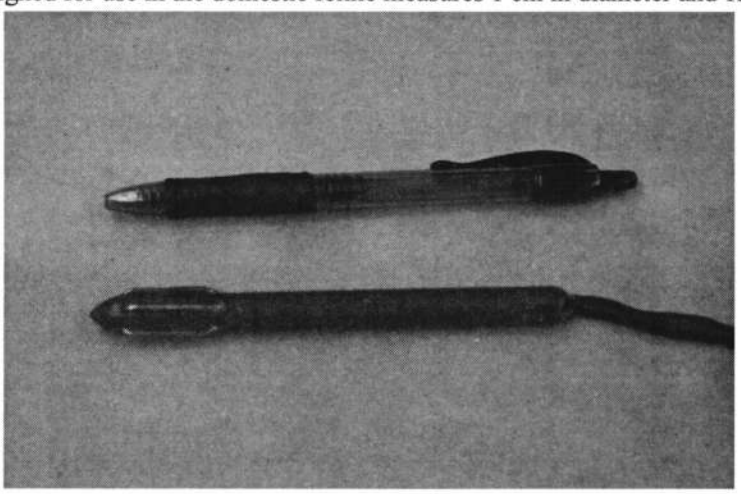
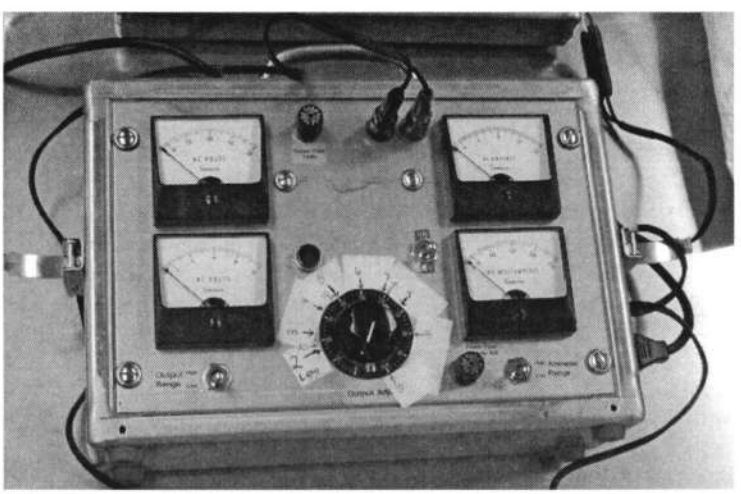


Figure 2. Several models of electroejaculators are available commercially. This one is produced by P-T Electronics (Boring, OR) and features a manual rheostat that offers complete control of the stimuli provided.



The tom's response to the stimuli should be monitored and the probe location adjusted accordingly. During the stimulation, both hind limbs typically would extend symmetrically. If they are not extending, or if one extends more than the other, confirm that the probe is in contact with the rectal wall, and that the electrodes are on ventral midline.

As an alternative to an ejaculate, collection of epididymal sperm by flushing post-castration or postmortem followed by cryopreservation has been described.[2-4] Research with this technique in the domestic cat has provided a model for the preservation of genetic material from endangered feline species.[5]

Ejaculated sperm for morphologic evaluation may be collected by aspiration or lavage of the queen's vaginal vault following mating. Collection by this method may also be useful to rule out azoospermia. Because retroejaculation is common in the tom, cystocentesis and analysis of the urine after ejaculation may yield enough sperm cells for a limited analysis but both sperm motility and morphology are likely to be compromised.

Semen evaluation

Following collection, the volume of the ejaculate is recorded, and the sample is immediately extended 1:1 using a slow, drop-wise addition with mixing of a suitable medium (Ham's F-10 with 25 mM Hepes, 1mM pyruvate and glutamine, penicillin/streptomycin/neomycin, and 5% fetal bovine serum). Motility (total and progressive) should be recorded by estimation under low power microscopy on a warmed microscope slide, or using a computer assisted analysis (CASA) calibrated for feline spermatozoa. Concentration is determined using a hemacytometer at a 1:100 dilution. The Nucleocounter® (Chemometec, Allerød, Denmark) has been used clinically to determine feline sperm concentration but requires at least 10 µL of sample. The low volume of the feline ejaculate often precludes routine use of this instrument. Volume of the ejaculate and sperm concentrations will vary between cats and collection method. The reported ranges for samples obtained using electroejaculation are 0.001-0.7 mL and 0.05-153 million sperm per ejaculate.[6]

Evaluation of sperm morphology is an integral part of the semen evaluation. Teratospermia in the domestic cat has been defined as less than 40% morphologically normal spermatozoa.[7] There is a very high degree of teratospermia in many of the exotic feline species studied, complicating genetic preservation.[8] Teratospermia is also observed in small populations of cats where inbreeding has occurred.[8] A single generation of inbreeding (offspring bred to parent) produced male offspring with less than 15% morphologically normal sperm compared to 55% morphologically normal sperm in control animals, indicating that loss of genetic diversity leads to increased teratospermia in as little as one generation.[8] The degree of teratospermia and the nature of the defects present affect the post-thaw survival of cryopreserved sperm, and the freezing method may need to be adjusted to compensate for these defects. For example, rapid cooling of semen collected from teratospermic cats resulted in larger number of damaged acrosomes compared to semen collected from normal controls. The number of damaged acrosomes is decreased when using a slower cooling rate.[9]

In other species, the number of morphologically normal sperm in an ejaculate and motility of the sperm are often closely correlated. In the cat, many teratospermic ejaculates demonstrate adequate motility (greater than 70%), in spite of low number of morphologically normal spermatozoa. Teratospermic cats appear to compensate for the lower number of morphologically normal spermatozoa by increasing spermatogenesis and frequency of copulatory activity. These strategies may improve breeding success by allowing an adequate number of normal sperm to be deposited into the tract.[8] Other measured parameters of the ejaculate may include pH, osmolality, membrane integrity, sperm chromatin structure, bacterial culture, or seminal plasma chemistry.

Cryopreservation

Successful cryopreservation of feline semen was first reported in 1978 by Platz, et al. with a conception rate of 11% following intravaginal insemination.[10] Subsequent research has compared various cooling rates, extenders, cryoprotectants, and techniques to achieve the optimal post-thaw motility for several feline species. For the domestic cat, TEST yolk buffer comprised of TES buffer, Tris lactose, and 20% egg yolk is an effective extender.[11] Glycerol at a final concentration of 4-5% is a commonly used as a cryoprotectant. Feline sperm appear to be sensitive to glycerol, and higher concentrations result in lower post-thaw sperm motility.[12]

Appropriate cooling rates optimize post-thaw sperm quality. Using an ultra-rapid (14 °C/min) or a rapid (4 °C/min) cooling rate from room temperature to either 0 or 5 °C caused a significant decrease in the number of intact acrosomes, thereby affecting the post-thaw semen quality in teratospermic cats. This effect was diminished when a slower cooling rate of 0.5 °C/min was used.[9] A comparison of five freezing rates indicated that a rate of 3.85 °C/min from 5 °C to -40 °C resulted in the greatest post-thaw motility and lowest number of damaged acrosomes.[13]

The authors use a protocol designed and tested by Pukazhenthi, et al. at the Smithsonian National Zoological Park.[14] Following collection, the ejaculate is immediately extended 1:1 in Ham's F-10 with Hepes. An

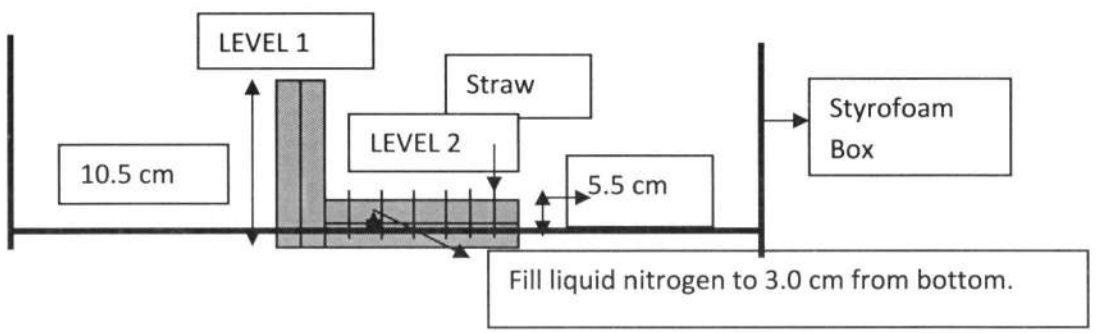
aliquot of the extended sample is used to obtain an initial motility and concentration using a hemacytometer. The remainder of the ejaculate is centrifuged at 300 x g for 8 minutes. The supernatant is removed. The resulting sperm pellet is re-suspended in a quantity of TEST medium (Refrigeration Medium; Irvine Scientific, Santa Ana,CA) sufficient to achieve a concentration of 100-120 x 10^6 motile sperm/mL (no fewer than 50 x 10^6 motile sperm/mL).

Feline spermatozoa appear to require a lower concentration of glycerol.[12] A final concentration of 4% glycerol is used during the final cryopreservation step. Initially, TEST-Freezing Medium (12% glycerol) is diluted to an 8% solution by combining two parts of TEST-Freezing Medium with one part TEST-Refrigeration Medium (0% glycerol). An equal volume of this 8% glycerol medium is added to the sperm solution in a three step process: add one-fourth of the total volume to the ejaculate, wait five minutes, add another one-fourth of the total volume, wait five minutes, then add the remaining volume. This results in a final glycerol concentration of 4%, and a final concentration of approximately 50-60 million motile spermatozoa/mL. The ejaculate is loaded into 0.25 mL straws and sealed. If loading less than 0.25 mL in a straw, a small amount of TEST medium with 4% glycerol can be loaded first, followed by an air bubble, then the sperm suspension. Straws are placed in a sealed plastic bag and submerged in a room temperature water bath. A plastic bottle (a squirt bottle or 0.5 L drinking bottle) can function well as a water bath. The water bath containing the straws is placed in a refrigerator or in a cooler with ice packs for two to three hours, until the water temperature reaches 5 °C. Once reaching 5 °C, the extended semen in the loaded straws is ready to freeze.

The procedure can be modified if working in a laboratory with a "cold room." The ejaculate is initially processed as above, but freezing medium containing glycerol is not added until after the cooling step. The processed ejaculate (in Refrigeration Medium) is placed in a room temperature water bath (300-350 mL) and placed in the cold room until the water bath reaches 5 °C. An equal volume of 8% TEST-Freezing Medium is prepared and cooled with the ejaculate. After reaching 5 °C, the freezing medium is added in a stepwise fashion (as described above) and the extended semen is loaded into cooled straws (5 °C) immediately prior to freezing.

Semen is frozen in a two step liquid nitrogen vapor method (Figure 3). Initially, straws are placed on a rack 7.5 cm above the liquid nitrogen (-30 °C) for one minute. The straws are then lowered to a second rack 2.5 cm above the liquid nitrogen (-130 °C) and held there for an additional one minute before plunging into the liquid nitrogen and transferred to canes for long term storage. To thaw, straws are removed from liquid nitrogen storage, held in the air for ten seconds, and transferred to a 37 °C water bath for 30 seconds. Immediately following thawing, the straws are thoroughly dried, opened, and the semen placed in a vial. The thawed semen is diluted in a drop wise fashion with an equal volume of warmed Ham's F-10 medium with Hepes buffer and 5% fetal calf serum. Following dilution, the sample is centrifuged at 300 x g for 8 minutes. The supernatant containing the egg yolk, glycerol, and diluent is immediately removed, and the pellet re-suspended in 100-200µL of the Ham's F-10 medium prior to insemination.

Figure 3: Diagram depicting the measurements for the two step freezing process. Two stainless steel test-tube racks of appropriate size; 1 placed on side, 1 placed flat, can be secured together with plastic ties. Pour adequate liquid nitrogen into styrofoam box and place the steel test-tube rack apparatus inside. Replace lid and allow to equilibrate prior to placing straws onto top level.



Use of cryopreserved sperm

Prior to insemination, estrus queens should be given an ovulation induction agent. A single dose of 100 IU human chorionic gonadotropin (hCG) or 25 µg gonadotropin-releasing hormone(GnRH; Cystorelin®, Merial, Duluth, GA) IM successfully induces ovulation approximately 25-27 hours after administration.[15] Queens are usually inseminated between 28-30 hours after ovulation induction.[16,17] When using frozen-thawed semen, intrauterine insemination is preferred to intravaginal insemination.[16,17] The birth of kittens following intravaginal

insemination of frozen-thawed semen has been reported, but success rates are low.[10] More recent studies failed to produce pregnancies following intravaginal insemination with frozen-thawed spermatozoa.[16,17] Intrauterine insemination is easily and quickly accomplished by laparotomy. A benefit to this procedure is it allows visualization of the uterus and ovaries. When inseminating at 30 hours after ovulation induction, corpora lutea can be observed, confirming ovulation has occurred.[17] A pregnancy rate of 57% (8/14 queens) following surgical insemination with 50 x 10^6 frozen-thawed spermatozoa has been reported.[18] Additionally, a 27% pregnancy rate (3/11 queens) following insemination with 5 x 10^7 frozen-thawed epididymal spermatozoa was achieved.[19] A third study resulted in a pregnancy rate of 75% (6/8 queens) in 2009 using approximately 40 x 10^6 motile spermatozoa.[17] Laparoscopic intrauterine insemination has been reported as an alternative to a laparotomy.[15] However, this study indicated that pre-ovulatory anesthesia may affect ovulation. Fewer corpora lutea, decreased embryo recovery, and reduced pregnancy rates were reported in cats that underwent laparoscopic intrauterine insemination under general anesthesia prior to ovulation compared to queens undergoing the same procedure performed after ovulation suggesting that it may be beneficial to perform surgical intrauterine inseminations after ovulation.[15]

Nonsurgical, transcervical insemination has been reported. Seventeen attempts resulted in 12 successful cervical catheterizations, four unsuccessful attempts, and one vaginal fornix penetration.[20] Several methods and variations of this technique have since been described.[21] Success rates with transcervical insemination are dependent on operator experience.

Summary

Collection of semen from the domestic cat can be accomplished with the necessary equipment. Evaluation of the ejaculate is similar to evaluation in other species. The cat often has a higher incidence of teratospermia due to the reduced genetic pool available in many catteries, research colonies, and exotic species. In natural breeding situations, cats appear to compensate for teratospermia by increased numbers of matings per estrus. Spermatozoal defects adversely affect the quality of the semen following cryopreservation. By adjusting the cooling rate and concentration of glycerol, many teratospermic cats have been successfully cryopreserved. The use of cryopreserved sperm can result in acceptable pregnancy rates if semen is placed directly into the uterus.

References

1. Zambelli D, Cunto M, Prati F, et al: Effects of ketamine or medetomidine administration on quality of electroejaculated sperm and on sperm flow in the domestic cat. Theriogenology 2007;68:796-803.
2. Tebet JM, Martins MI, Chirinea VH, et al: Cryopreservation effects on domestic cat epididymal versus electroejaculated spermatozoa. Theriogenology 2006;66:1629-1632.
3. Toyonaga M, Sato Y, Morita M, et al: The qualities of cryopreserved epididymal sperm collected from feline epididymides stored at low temperature. J Vet Med Sci 2010; Jan 22 [Epub ahead of print].
4. Siemieniuch MJ, Woclawek-Potocka I: Assessment of selected quality parameters of epididymal cat (Felis catus s. domestica, L. 1758) sperm using flow cytometry method and computer assisted sperm analyser. Reprod Domest Anim 2008;43:633-637.
5. Pukazhenthi B, Comizzoli P, Travis AJ, et al: Applications of emerging technologies to the study and conservation of threatened and endangered species. Reprod Fertil Dev 2006;18:77-90.
6. Johnston SD, Root-Kustritz MV, Olson PN: Canine and feline theriogenology. Philadelphia: Saunders; 2001. p. 112-121.
7. Wildt DE: Endangered species spermatozoa: diversity, research, and conservation. In: Bartke A, editor. Function of somatic cells in the testis. New York: Springer-Verlag, Inc; 1994. p. 1-24.
8. Pukazhenthi BS, Neubauer K, Jewgenow K, et al: The impact and potential etiology of teratospermia in the domestic cat and its wild relatives. Theriogenology 2006;66:112-121.
9. Pukazhenthi B, Santymire R, Crosier A, et al: Challenges in cryopreserving endangered mammal spermatozoa: morphology and the value of acrosomal integrity as markers of cryo-survival. Soc Reprod Fertil Suppl 2007;65:433-446.
10. Platz CC, Wildt DE, Seager SW: Pregnancy in the domestic cat after artificial insemination with previously frozen spermatozoa. J Reprod Fertil 1978;52:279-282.
11. Luvoni GC: Gamete cryopreservation in the domestic cat. Theriogenology 2006;66:101-111.
12. Nelson K, Crichton E, Doty L, et al: Heterologous and homologous fertilizing capacity of cryopreserved felid sperm: a model for endangered species [abstract]. Theriogenology 1999;51:290.
13. Zambelli D, Caneppele B, Castagnetti C, et al: Cryopreservation of cat semen in straws: comparison of five different freezing rates. Reprod Domest Anim 2002;37:310-313.
14. Howard J, Zhang Z, Desheng L, et al. Male reproductive biology in giant pandas in breeding programmes in China. In: Wildt D, Zhang A, Zhang H, et al., editors. Giant Pandas: Biology, Veterinary Medicine and Management. Cambridge: Cambridge University Press; 2006. p. 159-97.
15. Howard JG, Barone MA, Donoghue AM, et al: The effect of pre-ovulatory anaesthesia on ovulation in laparoscopically inseminated domestic cats. J Reprod Fertil 1992;96:175-186.
16. Chatdarong K, Axner E, Manee-In S, et al: Pregnancy in the domestic cat after vaginal or transcervical insemination with fresh and frozen semen. Theriogenology 2007;68:1326-1333.
17. Villaverde A, Melo C, Martin I, et al: Comparison of efficiency between two artificial insemination methods using frozen-thawed semen in domestic cat (Felis catus): artificial insemination in domestic cats. Anim Reprod Sci 2009;114:434-442.
18. Tsutsui T, Tanaka A, Takagi Y, et al: Unilateral intrauterine horn insemination of frozen semen in cats. J Vet Med Sci 2000;62:1247-51.

19. Tsutsui T, Wada M, Anzai M, et al: Artificial insemination with frozen epididymal sperm in cats. J Vet Med Sci 2003;65:397-399.
20. Hurlbut SL BM, Kraemer DC: The feasibility of transcervical catheterization and nonsurgical embryo collection in the domestic cat. Theriogenology 1988;29:264.
21. Zambelli D, Cunto M: Transcervical artificial insemination in the cat. Theriogenology 2005;64:698-705.

Pregnancy termination in the dog–an overview and case presentations

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Abstract

Several methods are available for termination of unwanted pregnancy in the bitch. Ovariohysterectomy is recommended for bitches not intended for breeding. In valuable breeding bitches, various pharmacological products have been used. Use of estrogens for prevention of pregnancy following accidental or undesired mating (mismating) is not recommended. The majority of bitches presented for abortifacient therapy are not pregnant. Therapy should only be initiated after pregnancy confirmation by ultrasonography or serum relaxin assay. Dinoprost trometamine is the most effective abortifacient when used after 25 days of pregnancy. Progesterone antagonists (e.g., mifepristone) and dopamine agonists (e.g., bromocriptine and cabergoline) are successfully used for pregnancy termination. Most of these products are not approved or available for use as canine abortifacients in the U.S.

Keywords: Canine, bitch, pregnancy termination, prostaglandin $F_2\alpha$, progesterone antagonists

Introduction

Several protocols are described for termination of unwanted pregnancy in the bitch. Ovariohysterectomy (OHE) is recommended for bitches not intended for breeding. In valuable purebred breeding bitches, various pharmacological products have been used. Comprehensive reviews on the topic are published[1] and more recent studies[2] have contributed to our understanding of canine pregnancy and its termination.

The objectives of the present paper are to review the current literature on the topic and provide a detailed description of clinical cases seen by the author.

Prevention of pregnancy following accidental or undesired breeding

Use of estrogens as immediate treatment for accidental or undesired mating is not recommended[3] for the following reasons:

- Many unintentionally mated bitches do not conceive
- No dose of estrogen is established to be efficacious and safe
- Potential side-effects of estrogens include pyometra and bone marrow suppression
- Other therapies such as prostaglandin $F_2\alpha$ (PGF) are available for pregnancy termination.

As mentioned above, many unintentionally mated bitches do not become pregnant. Out of 48 privately-owned bitches evaluated 30 to 35 days after a single unplanned breeding, 30 (62%) bitches were determined not to be pregnant by abdominal ultrasonography (US) and 18 bitches were confirmed to be pregnant.[4] For management of mismating cases, an algorithm on management may be followed.[5] The mechanisms of action of estrogen when used to treat mismating are suggested to involve estrogen-induced closure of utero-tubal junction and prevention of embryo transport as well as a possible direct embryotoxic effect, based on studies in cats.[6]

Several estrogen treatment protocols with successful outcomes have been reported.[7] However, undesirable side-effects of estrogen administration such as pyometra and bone marrow suppression are reported. Pyometra developed in two out of eight dogs receiving estradiol cypionate (ECP) during diestrus.[7]

Case presentation

A one-year old Rottweiler, Tory, presented for persistent vaginal discharge for about five days. Tory was observed in early proestrus seven weeks prior to presentation. She was accidentally mated two weeks after the beginning of proestrus bleeding. Tory was treated with ECP by the attending veterinarian to prevent pregnancy. Following the treatment Tory remained in estrus for about three weeks. Proestrus bleeding stopped. Two weeks after treatment with ECP, dark red-brown, thick vaginal discharge was noticed by the client. Vaginal swabs taken by the attending veterinarian were submitted for culture yielded growth of *E. coli*. Tory was treated with a combination of amoxicillin trihydrate and clavulanate potassium (Clavamox®, Pfizer Animal Health, New York, NY). Tory was reportedly anorexic for about a week and had two episodes of urinary incontinence.

On presentation, Tory's rectal temperature, pulse and respiration rate were 38°C, 90 beats per minute, and 20 breaths per minute, respectively. A dark brown vaginal discharge was observed upon examination of the perineal area. On transabdominal US, the uterus was seen to be enlarged and fluid-filled; each uterine horn measured 2.5cm in diameter. The uterine wall was thickened and measured 6-8mm. A diagnosis of pyometra was offered; OHE was

recommended and was accepted by the client. The reproductive tract was examined after surgery and revealed multiple corpora lutea (CLs) on both ovaries and an enlarged uterus with mucopurulent contents. Tory's recovery from OHE was uneventful.

Termination of unwanted pregnancy

Progesterone (P4) is the only hormone required for pregnancy maintenance in the bitch as demonstrated by the ability to maintain pregnancy with exogenous progesterone after ovariectomy.[8] The sole source of P4 during pregnancy in the dog is from the ovarian CLs. Most of the methods suggested for pregnancy termination are based upon interrupting or interfering with the supportive role of P4 for pregnancy maintenance.

Case presentation

The following two bitches were presented for termination of unwanted pregnancy. The first case was a two-year old Airedale, Sunny, referred with a history of accidental breeding 37 days prior to presentation. The second case was a 1.5-year old Irish wolfhound bitch, Graniall. Approximately 50 days prior to presentation, she escaped from her owner's supervision for one evening. At presentation, Graniall was beginning to exhibit mammary gland enlargement. Both bitches were apparently healthy with no concurrent medical problems.

Ultrasound examination revealed six to nine fetuses with normal heartbeats in Sunny and US and radiographic evaluation of Graniall showed nine living fetuses with normal conformation and skeletal mineralization consistent with late gestation. Both bitches were treated with PGF (Lutalyse®, Pfizer Animal Health, New York, NY), 0.1 mg/kg SQ at eight hour intervals. Sunny received a total of ten doses. Graniall received 12 doses without aborting, and subsequently the dose was increased to 0.125 mg/kg SQ TID for nine additional doses.

In Sunny's case, one fetus still enclosed within an intact amniotic sac was aborted following the ninth PGF injection. Examination with US after the tenth treatment revealed the uterus to be diffusely filled with echogenic material with no fetuses. These US findings were suggestive of a postpartum uterus. It was suspected that Sunny must have aborted and consumed the rest of the fetuses. Sunny's serum P4 decreased dramatically from 40.4 ng/ml before therapy to 5.4 ng/ml within 24 hours. Progesterone concentrations continued to fall to 1.23 ng/ml at the completion of the abortion.

In Graniall's case, all nine fetuses were aborted over a two day period, with the final abortion following the ninth injection at the higher dose. The average interval between aborted fetuses was about three hours (range 1 to 14.5 hrs). The maximum interval was required for the last fetus to be delivered. Serum P4 levels decreased from 64.80 ng/ml to 12.0 ng/ml within 24 hours after the first PGF treatment. Progesterone concentrations continued to decline to 3.63 ng/ml after the twelfth injection of 0.1 mg/kg PGF without triggering abortion. With the higher dose, serum P4 decreased further to 3.16 ng/ml and resulted in delivery of the first fetus. Progesterone concentrations continued to fall to 1.43 ng/ml at the completion of the PGF treatments.[9]

The side-effects of PGF treatment including hypersalivation, panting, diarrhea and occasional vomiting were noticed within 30 minutes after each injection in both bitches and diminished with subsequent injections. Intermittent, non-odorous, serosanguineous vaginal discharge was noticed inconsistently throughout the treatment in both bitches. Lactation also occurred in both bitches towards the end of the abortions.

Pregnancy termination with PGF

Natural PGF and its analogs are commonly used for pregnancy termination. Prostaglandin $F_2\alpha$ is luteolytic, and administration during diestrus causes a decrease in P4 production in the dog.[10] During pregnancy, administration of PGF results in P4 decline and ultimately fetal loss. Prostaglandin $F_2\alpha$ is also uterotonic and facilitates expulsion of uterine contents via myometrial contraction. In order to achieve complete evacuation of uterine contents, PGF needs to be administered IM or SC two or three times a day until the pregnancy is terminated.[1] The half-life of PGF is only a few seconds, therefore multiple injections are essential.[1] Treatment is continued until all fetuses are expelled as confirmed by US examination. Even though most of the abortions occur within five to seven days after the initiation of treatment, it may take longer to terminate the pregnancy in some cases[1] as observed in the case of Graniall.

The most commonly used PGF in the U.S. is dinoprost tromethamine. This product is marketed for use in large animals and its use in dogs is extra-label. A release form or statement to document owner consent is recommended.

Hospitalization of the patient is highly recommended in order to monitor the adverse side-effects and efficacy of the treatment. The author's clinical observation is that the side-effects (hypersalivation, panting, etc.) are decreased after the initial few injections. To prevent vomiting, it is recommended not to feed the bitch before

treatment; and to minimize abdominal cramps and discomfort, the bitch may be walked for five to ten minutes immediately after each PGF injection.

The synthetic PGF analog cloprostenol (Estrumate®, Intervet/Schering-Plough Animal Health, Summit, NJ) is highly potent but is not commonly used in the U.S. The reasons for its limited use include lack of dose-response and efficacy studies as well as lack of familiarity of small animal practitioners with the product which may lead to dosage errors that could be fatal.[1]

Pregnancy termination with progesterone antagonists

Progesterone antagonists are a group of synthetic steroids that bind to P4 receptors and prevent the action of endogenous P4. The anti-progestin mifepristone (RU486) was developed for human use and is available in some countries. Early trials conducted by Concannon, et al[11] demonstrated that pregnancy can be terminated with a dose of 2.5 mg/kg, BID, PO for 4.5 days beginning on day 32 of pregnancy. This treatment was safe and no side-effects similar to those seen with PGF therapy were observed. In that study, a powdered formulation of appropriate quantity was inserted into gelatin capsules for individual animals.

Aglepristone (RU 534), an injectable analog of RU486, was made available for veterinary use in France in 1996. In a recent study,[2] aglepristone administration to bitches during the mid-luteal phase markedly accelerated the luteolytic process accompanied by a parallel decline in ovarian blood flow as observed by US. Aglepristone, developed by Roussel Uclaf, is available in Europe under the name of Alizine®. Clinical studies showed no side-effects at the recommended dose, and make it a desirable method for pregnancy termination before day 35.[1,12]

Other methods for pregnancy termination

Dopamine agonists. In addition to luteinizing hormone, prolactin is a major luteotrophic hormone produced throughout the luteal phase in pregnant as well as in non-pregnant bitches.[13] Dopamine agonists, such as the ergot alkaloids bromocriptine and cabergoline, have strong dopamine D2-receptor agonist activity. Administration of these products has been shown to reduce prolactin concentrations resulting in luteolysis and a decrease in P4 production.[1]

Bromocriptine, (Parlodel®, Novartis Pharmaceuticals Corp., East Hanover, NJ) at doses of 0.1 mg/kg, PO or IM daily or BID, for six days has been shown to terminate pregnancy after day 30, but failed to do so when given earlier.[13] It is marketed for human use to treat hyper-prolactinemia, and is not approved for veterinary use in the U.S.

Cabergoline has been successfully used for pregnancy termination and is approved for veterinary use in Europe. Compared to bromocriptine, it is a more potent dopamine agonist, and is effective in terminating pregnancy when administered at mid-gestation or later.[14] Bitches were treated after day 40, at doses of 5 µg/kg, PO, for five days, or doses of 1.75 µg/kg, SC every two days for six days. Cabergoline effectively terminated pregnancy in all bitches treated.[15,16]

Corticosteroids. Dexamethasone administered beginning at mid-gestation can terminate pregnancy in dogs.[17] The common uses of dexamethasone in veterinary practice are for its anti-inflammatory and immuno-suppressive functions. Oral administration of dexamethasone is considered as an advantage, whereas the concerns include limited published information and lack of data on the effects on the adrenal glands.

Conclusion

Although induction of abortion may be achieved reliably with some of the treatments described above, the majority of bitches presented for abortifacient therapy are in fact not pregnant. Therefore therapy should not be initiated until pregnancy is confirmed by US or serum relaxin assay after 25 days from the last breeding. Natural PGF in an affective abortifacient after 25 days of pregnancy but its side-effects raise ethical issues. Antiprogesterone therapies offer the advantage of safety and no side-effects and are a desirable option where available. Ovariohysterectomy should be considered in bitches not intended for breeding. Estrogen therapy to prevent pregnancy following mating is not advised.

References

1. Wanke MM, Romagnoli S, Verstegen J, et al: Pharmacological approaches to pregnancy termination in dogs and cats including the use of prostaglandins, dopamine agonists, and dexamethasone. In: Concannon PW, England G, Verstegen III J, et al, editors. Recent advances in small animal reproduction. Ithaca (NY): International Veterinary Information Service, 2002; A1223.0802. Available from: http://www.ivis.org/advances/Concannon/wanke/chapter_frm.asp?LA=1

2. Polisca A, Scotti L, Orlandi R., et al: Aglepristone (RU534) administration to non-pregnant bitches in the mid-luteal phase induces early luteal regression. Theriogenology 2010; epub ahead of print. Available from: http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6TCM-501CG0X-3-9&_cdi=5174&_user=3419478&_pii=S0093691X10001810&_orig=browse&_coverDate=05%2F07%2F2010&_sk=999999999&view=c&wchp=dGLzVzb-zSkWA&md5=bd01a89e5615a34fdd91fde641796a19&ie=/sdarticle.pdf
3. Concannon PW, Meyers-Wallen VN: Current and proposed methods for contraception and termination of pregnancy in dogs and cats. J Am Vet Med Assoc 1991;198:1214-1225.
4. Feldman EC, Davidson AP, Nelson RW, et al: Prostaglandin induction of abortion in pregnant bitches after misalliance. J Am Vet Med Assoc. 1993;202:1855-1858.
5. Feldman EC, Nelson RW: Induced abortion, pregnancy prevention and termination, and mismating. In: Canine and feline endocrinology and reproduction. 3rd ed. St. Louis: Saunders; 2004. p. 835-851.
6. Herron MA, Sis RF: Ovum transport in the cat and the effect of estrogen administration. Am J Vet Res 1974;35:1277-1279.
7. Bowen RA, Olson, PN, Behrendt MD, et al: Efficacy and toxicity of estrogens commonly used to terminate canine pregnancy. J Am Vet Med Assoc 1985;186:783-785.
8. Concannon PW: Understanding and monitoring canine pregnancy. Proc World Small Anim Vet Assoc 2005. Available from: http://www.ivis.org/proceedings/wsava/2005/97.pdf
9. Memon MA, Myers NC, Agarwal RK, et al: Termination of unwanted pregnancy and premature luteolysis with prostaglandin $F_2\alpha$ in two dogs. Canine Pract 1993;18:5-10.
10. Concannon PW, Hansel W: Prostaglandin F2a induced luteolysis, hypothermia and abortions in Beagle bitches. Prostaglandins 1977;13:533-542.
11. Concannon PW, Yeager A, Frank D, et al: Termination of pregnancy and induction of premature luteolysis by the antiprogestagen, mifepristone, in dogs. J Reprod Fertil 1990;88:99-104.
12. Fieni F, Marnet PG, Martal J, et al: Comparison of two protocols with a progesterone antagonist aglepristone (RU534) to induce parturition in bitches. J Reprod Fertil 2001;Suppl 57:237-242.
13. Concannon PW, Weinstein R, Whaley S, et al: Suppression of luteal function in dogs by luteinizing hormone antiserum and by bromocriptine. J Reprod Fertil 1987;81:175-180.
14. Post K, Evans LE, Jochle W: Effects of prolactin suppression with cabergoline on the pregnancy of the bitch. Theriogenology 1988;29:1233-1243.
15. Onclin K, Silva LDM, Donnay I, et al: Luteotrophic action of prolactin in dogs and the effects of a dopamine agonist, cabergoline. J Reprod Fertil 1993;Suppl 47:403-409.
16. Onclin K, Silva LDM, Verstegen JP: Termination of unwanted pregnancy in dogs with the dopamine agonist, cabergoline, in combination with a synthetic analog of PGF2-alpha, either cloprostenol or alphaprostol. Theriogenology 1995;43:813-822.
17. Zone M, Wanke M, Rebuelto M, et al: Termination of pregnancy in dogs by oral administration of dexamethasone. Theriogenology 1995;43:487-494.

Maternal and fetal abnormalities during gestation in the cow

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Introduction

The focus of this presentation is to describe several noninfectious complications of gestation in the cow. The emphasis will be on the diagnosis, prevalence, and prognosis of various accidents of gestation. Included will be hydrallantois, hydramnios, cervico-vaginal prolapse, and torsion of the uterus for the cow, and Schistosomus reflexus, hydrocephalus, incomplete twins, mummification and maceration for the fetus. Illustrations are included via a direct reference the Bovine Reproduction Guide, a web atlas.

Keywords: Abnormal gestation, hydrallantois, hydramnios, cervico-vaginal prolapsed, uterine torsion

Hydrallantois/hydrops allantois

Excessive accumulation of allantoic fluid is seen sporadically in dairy as well as beef cattle. Hydrops allantois may be progressive after mid-gestation. As much as a 10-fold increase in allantoic fluid volume, up to 200 liters, has been reported[1] versus a normal near-term volume of 8 to 15 liters.

Cows show bilateral distension of the abdomen [bags48.jpg].[2] They are distressed, anorectic and have no rumen activity due to compression of the rumen. Dehydration and constipation follow and eventually the thin, distended cows become recumbent. Palpation per rectum reveals a very tight uterine wall [bags64.jpg],[2] too tight to identify fetal parts or placentomes.

Placental dysfunction is evident by the occurrence of adventitious placentation which is characterized by a reduced number of placentomes (normal range 75 to 120) and the development of a more primitive villous placentation [bpla33.jpg].[2] Nutritional deficiencies have been reported to cause hydrops allantois near Zacatecas, Mexico in Criollo cattle owned by small holders, and raised on poor soil during the long, severely dry season from December to May, with a prevalence of up to 30%.[3]

Salvage slaughter is generally recommended because of the underlying adventitious placentation which is a permanent alteration of the caruncular structures of the endometrium. Gradual drainage by repeated trocarization [bags53.jpg][2] is possible when supported by oral fluid therapy. Parturition can be induced if the cow is reasonably close to term (within 2 to 3 weeks), provided she is simultaneously treated with large amounts of electrolytes per os. When the accumulation of fluids is the result of nutritional deficiencies, as reported in Mexico, improved nutrition will help if the condition is diagnosed early. The prognosis for future fertility is guarded in cases of adventitious placentation, and favorable when due to nutritional causes.

Hydramnios/hydrops amnii

Excessive accumulation of amniotic fluid can be the result of fetal anomalies such as impaired deglutition or renal dysgenesis or agenesis. Hydramnios is a very rare condition. The increase of amniotic fluid is gradual. When viewed from the rear, a cow with hydramnios has a pear-shaped abdomen [bags58.jpg].[2] Hybrid fetuses resulting from the mating of an American Bison bull with a domestic cow have caused hydramnios as a result of an immunologically compromised placenta. The fetus is invariably defective and nonviable. Placentation is normal. Parturition and labor may be induced with glucocorticoids and prostaglandins, or the cow may be allowed to go into labor spontaneously. The prognosis for future breeding of the dam, to a different bull, is good.

Cervico-vaginal prolapse

Elevation of the plasma estrogen concentration during late gestation predisposes the cow to cervico-vaginal prolapse. Contributing factors are pluriparity, obesity, *Bos indicus* breeding, large calves, and occasionally hilly terrain.

Development of the prolapse is progressive and starts with the exposure of some of the vaginal mucosa. The prolapsed mass moves in and out as the cow gets up and lies down. The membranes dry out upon exposure and become irritated which leads to straining and greater exposure of the mass. Edema is the result of, and leads to further circulatory impairment and more swelling. Ultimately the cervix and occasionally the bladder may become involved.

Diagnosis is obvious, based on the appearance of an angry looking soiled mass protruding from the vulva [bags97.jpg].[2] However, the mass may be mistaken for fetal membranes containing bloody fluids, hematoma of the vulva, cystic vestibular glands [bags91.jpg][2] and tumors.

Treatment depends on the severity of the condition and varies from simply elevating the hindquarters of the cow with a platform in a tie stall, to the placement of retention sutures or prolapse pins in the vulva [bags79.jpg].[2] In non-pregnant animals a deep purse string suture (Buhner) can be placed under epidural anesthesia [bob251 to bob268.jpg].[2]

The prognosis also depends on the severity. As most of the prolapses occur during late gestation, the cow must be observed for signs of impending parturition to allow for timely removal of the prolapse pins or sutures. There is no correlation with prolapse of the uterus after parturition. Vaginal prolapse is like to recur during the next pregnancy, whereas uterine prolapse usually does not recur.[4]

Uterine torsion

Cows are predisposed to torsion of the uterus because the broad ligament is attached ventrally along the lesser curvature of the uterus. This leaves the greater curvature free. In *Bos indicus* the ventral attachment changes from ventral at the body to dorsal at the tip of the horn. As cows get up on their hind legs first the (gravid) uterus is temporarily suspended. The broad ligament is looser and longer in pluriparous cows. The abdomen is capacious especially when the rumen is relatively empty. Strong fetal movements and poor maternal muscle tone further contribute to torsion of the uterus. In 89% of the cases the weight of the fetus is above the mean.[5]

When the uterus is rotated, there is evidence of abdominal pain and discomfort due to stretching of the broad ligament. Anorexia, rumen stasis, constipation, increased pulse and respiration are usually present.

The diagnosis is based on a history of advanced pregnancy. Per rectum, the orientation of the broad ligaments is distinctly different. Depending on whether the torsion is to the left or the right, the respective broad ligament is pulled tight across the uterus. Spiral folds can be palpated per vaginam. Most torsions are to the left (counter clockwise), as, in general, the uterus rolls toward and over the nongravid horn. About 60% of all pregnancies in the cow are in the right horn. Thirty-four percent of uterine torsions occur anterior to the cervix and there is no vaginal involvement in those cases. Forty-five to 90 degree torsions are common and are not diagnosed per se. Of the more severe torsions, 20% are 90 to 180 degrees, 57% are 180 to 270 degrees, and 22% are 270 to 360 degrees.[5] Depending on the degree of torsion, the fetus may be in dorso-pubic presentation. With severe torsion circulatory embarrassment occurs.

Treatment depends on the degree of the torsion. With rotations of 90 degrees or less, the fetus can frequently be manually rocked into a normal dorso-sacral position when the cow is in labor and the legs are presented into the birth canal. Greater rotations can be corrected by rolling the cow around the fetus which is kept in placed by a plank in the flank [bags98.jpg].[2,6] Briefly, the cow is cast with ropes to lie on the side in the direction of the torsion. A long plank is placed in the paralumbar fossa of the cow and an adult person stands on the plank above the paralumbar fossa. Next the front legs of the cow are tied together and likewise the hind legs and they are pulled up and over the recumbent cow. In intractable cases a cesarean section must done to deliver the fetus, suture the uterus, and manually untwist the uterus. The prognosis depends on the degree of severity and largely on the extent of vascular compromise. The latter may cause rupture of the fragile uterus [bags95.jpg].[2]

Fetal complications

The occurrence of congenital anomalies and fetal monsters is uncommon in the bovine, which makes it difficult to study their etiology. Two major categories are genetic influences and toxic effects. A short, random list of conditions that may lead to dystocia is hereby presented.

Schistosoma reflexum literally means split body that is bent back on its self [bter75.jpg].[2] This aptly describes a fetus with a wide open abdominal cavity and free flowing viscera, and a vertebral column that has doubled back on itself [bter10.jpg].[2] These fetuses survive until term and then present a problem in delivery because of the awkward conformation. The diagnosis presents a challenge when encountered for the first time. The condition is rare and the etiology is obscure. A cesarean section is contraindicated because of the severity of the abnormality. A single cut with the fetatome through the curvature of the spine is sufficient to reduce size of the two halves. If the fetus is alive, its umbilical cord may be severed prior to the fetotomy.

Perosomus elumbis is characterized by a lack of development of the hindquarters, either a total absence of the pelvis and hind legs [bter55.jpg],[2] or a rudimentary pelvis with short hind limbs [bter56].[2] The latter may prevent spontaneous delivery. They can be removed by fetotomy.

Hydrocephalus is a condition marked by excessive cerebrospinal fluid resulting in dilation of the cerebral ventricles. This may result in enlargement of the cranium and atrophy of the brain [bter29.jpg].[2] The grossly enlarged skull may be too large to pass through the birth canal, hence cause a dystocia. A cesarean section is contraindicated due to the condition of the fetus, which can readily be delivered after the soft, fluctuant skull [bter22.jpg][2] has been incised, and drained.

Identical twins are formed when one or more of the early totipotent cells become separated from the inner cell mass of the embryo, and develop into two individuals. When the separation is incomplete two partially developed calves and conjoined twins result. At other times, only certain regions of the twins are duplicated, as in the cases of a calf with two heads (dicephalus) [bter39.jpg],[2] or a calf with two sets of hindquarters. Other examples include the occurrence of a parasitic (extra) limb [bter58.jpg].[2] Extreme examples of incomplete twins are the formation of a globosus amorphus, a spherical mass, with its own vascular connection to the placenta of its normal twin [bter64.jpg].[2]

Mummification

Mummification of the fetus is a curious and interesting event which fortunately does not occur very often. The etiology of mummification is generally obscure. There are several prerequisites for the process of mummification, the fetus must be dead, there must be no air (oxygen) in the uterus (the cervix must be tightly closed), and no bacteria should gain access to the uterus via its blood supply. This makes certain viruses suspect (enteroviruses, BVD) which can kill a fetus quickly without causing further contamination and irritation.

Mummification is most common at end of the first and the beginning of the second trimester of pregnancy in the cow [bags75.jpg].[2] After the fetus dies, the fetal fluids are gradually resorbed and the fetus itself becomes progressively more dehydrated. The wall of the uterus shrinks tightly around the fetus, ultimately even into the empty eye sockets as the eyeballs have completely shriveled up. The caruncles disappear completely as well. The fetus slowly compacts and attains a leathery texture. The entire process takes several weeks depending on the age of the fetus at the time of its death.

Meanwhile the cow behaves normally but she does not cycle. Frequently the first indication that something is wrong is when the cow shows no udder development near the time she is expected to calve, and indeed she fails to calve. Examination at that time by transrectal palpation reveals a uterus that is devoid of fluids and that is drawn tightly around a small, firm fetus with a bird-like head. The empty sockets are usually readily recognized.

Treatment is relatively simple though questionable for economic reasons, hence the cow should be sent to slaughter. The cow is generally already dry and it will require a minimum of ten months for her to conceive again and deliver her next calf. When given a single injection of 25 mg prostaglandin $F_2\alpha$ i.m. she will come into heat in 3 to 5 days at which time her cervix relaxes, her uterus contracts and the mummy will be presented in the birth canal. Because of its sticky, dry nature it is best to examine the vagina 3 to 5 days after the injection to check for the presence of the odorless, rat- to cat-size mummy and aid in the delivery if necessary. The occasional large mummy for which the uterine contractions are too weak and for which the cervix does not dilate sufficiently, may need to be delivered by cesarean section [bags37.jpg].[2] After expulsion of the mummy the uterus quickly regresses in size. Its lumen is not contaminated, the caruncles are already involuted, and hence the cow may be bred back at the next heat with a good chance of conception.

Fetal maceration

While fetal mummification occurs when the fetus quietly dies in the uterus in the absence of air and bacterial contamination, and the cervix remains tightly closed, fetal emphysema and maceration occur when the cervix is open and miscellaneous bacteria invade the uterus from the vagina. Fetal maceration follows incomplete abortion but it is not common. The latter may be the result of an only partially dilated cervix or the abnormal presentation of a fairly dry fetus which causes it to be retained in the uterus.

With the dead fetus incubated at body temperature, bacterial multiplication is rapid and the fetus putrefies. Initially it becomes distended with gas and it subsequently decomposes. The wall of the uterus becomes thick and surrounds the disintegrating fetus like a capsule, as if to wall off an abscess [bags32.jpg].[2] This helps explain why the cow does not become seriously ill. After about the third month of gestation fetal bones resist maceration. Sharp pointed bones such as the fetal ribs may deeply embed themselves in the uterine wall. Occasionally a bone perforates the uterine wall and becomes encapsulated by adhesions. The rare finding of a small bone free in the abdominal cavity of a cow at slaughter can be explained in this manner.

Meanwhile, the cow may have shown only vague signs of intermittent straining accompanied by a foul, grayish-red vaginal discharge which may contain some of the smaller bones. She may run a fever, go off feed, and act depressed. These changes are usually noticed in the lactating cow as her milk production also drops, but they are easily overlooked in heifers, dry cows, or beef cows. The diagnosis is readily made by palpation per rectum. The uterus feels thick-walled and firm, fluctuation is largely absent, and in advanced cases crepitation of the fetal bones can be felt [bags32.jpg].[2] There is usually also a slight, purulent vaginal discharge.

The prognosis for future fertility of the cow is very poor because of the damage done to the uterine wall. For economic reasons treatment is therefore not advisable. The cow should be sent to slaughter. If the individual

value of the animal nevertheless warrants treatment, prostaglandins can be used to evacuate the contents of the uterus. It must be realized that some bones may be partially embedded in the wall or lodged sideways preventing their expulsion. As a last resort such bones can be removed surgically [bags67.jpg].[2] Surgery is best performed via a midline incision in the abdomen to provide optimal access to the small, contaminated uterus.

References

1. Ŝloss V, Dufty JH: Handbook of bovine obstetrics. Baltimore: Williams and Wilkins; 1980. p. 89.
2. Visual Guide to Theriogenology [http://drostproject.org]. Bovine Reproduction Guide, under the heading of Accidents of Gestation.
3. Flores F, Valencia J, Fernandez Baca S, et al: Hidropesía de las Membranas Fetales de los Bovinos. Proc Congr Mundial de Buiatrica; 1977; p.121-122.
4. Risco CA, Reynolds JR, Hird DE: Uterine prolapse and hypocalcemia in dairy cows. J Am Vet Med Assoc 1984;185:1510-1513.
5. Frazer GS, Perkins NR, Constable PD: Bovine uterine torsion: 164 hospital referral cases. Theriogenology 1996;46:739-758.
6. Roberts SJ: Veterinary obstetrics and genital diseases (theriogenology). 3rd ed. Woodstock (VT): Published by the author, 1986. p. 225

Images

Illustrations of the various conditions may be viewed in the Bovine Reproduction Guide under the heading Accidents of Gestation.[2] To view an individual image, select Search on the Home Page and enter the file name (e.g. bags48.jpg), or enter the name of the condition.

Client education.

Options for training personnel on the farm in reproductive management

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Introduction

Dairy farm workers, and to a lesser extent employees on a beef cattle operation, play a major role of success in the areas of artificial insemination, heat detection, pregnancy diagnosis, obstetrics and even advanced reproductive technology. This requires a team effort whereby the veterinarian serves as the coach. With the motto that a picture is worth 1,000 words, s/he can use the Visual Guide to Bovine Reproduction[1] to illustrate the various processes.

The veterinarian needs to coordinate the activities of the farm workers in conducting a successful breeding program and measure this success with early pregnancy diagnosis. The team must be proactive during the calving season to minimize calf losses and injuries to the dam. The latter will optimize subsequent fertility. Teaching farm workers about accidents during gestation will stimulate their general interest and motivate them. Success with embryo transfer and in-vitro fertilization programs is based on the quality of the basic reproductive management program.

Emphasis will be placed on the subject of obstetrics to serve as a model for an on-farm training program.

Keywords: Client education, artificial insemination, pregnancy diagnosis, obstetrics,

Artificial insemination

While veterinarians have handed the task of actual insemination over to technicians, it is important that inseminator understand the basic anatomy of the reproductive tract as well as the timing of ovarian activity. It is recommended that the ovaries are not routinely palpated near the time of ovulation. Appreciating the role and function of the follicles and the corpus luteum and the critical sequence of events is helpful (Subject: Reproductive Technology).[1]

The importance of accurate heat detection is currently underestimated and underutilized on many farms. Teaser bulls with surgically deviated penises and equipped with chin-ball markers are excellent estrus detectors (Subject: Estrus Detection).[1]

Pregnancy diagnosis

The first suggestion, after insemination, that the heifer or cow is pregnant is her failure to come back in heat. Physical changes in the uterus can be palpated per rectum. Asymmetry between the uterine horns, especially in heifers, starting around 4 weeks of gestation is the first indication. This is followed by the detection of the presence of fluid. The chorio-allantoic membrane slip can be felt around 5 weeks, and shortly after the presence of the amniotic vesicle.[2] Experience and a gentle touch are required during these early stages prior to 6 weeks. Progressive development of the fetus is illustrated under the Subject: Pregnancy.[1]

Ultrasonography has further advanced the technique and the accuracy of early pregnancy diagnosis (Subject: Ultrasonography).[1,3] Early pregnancy can also be determined by the presence of pregnancy associated glycoproteins in the blood of pregnant cows, approximately 25 days after insemination.[4]

Calving management

Calving management has traditionally been approached in a passive manner. On large dairy farms the 24/7 supervision and care of the calving pen is generally delegated to employees with varying degrees of knowledge and skill in obstetrics. This first line of defense is important because early intervention not only prevents calf losses but also protects subsequent fertility. However, too often the first reaction when a cow is in labor is to immediately hook onto the calf and start pulling, frequently with a calf puller.

The person assisting must be clean and should follow some simple guidelines to determine whether a calf can be pulled with reasonable force, or whether the delivery will require more drastic measures such as use of a calf puller, cesarean section or fetotomy.

A secondary problem is that calving difficulties rarely occur at a predictable or convenient time, when there is adequate help. Unfortunately, both haste and delays lead to injuries to the calf or to the dam, or to both.

Signs of calving

Progressive udder development is one of the earliest signs of the approach of calving. Early enlargement occurs in heifers during the fourth month of pregnancy. In cows, enlargement of the udder may not become apparent until two to three weeks before calving. Finally, the teats become turgid and lose their wrinkles. The lips of the vulva also become larger and softer, and lose their wrinkles. As the pelvic ligaments relax, the tail head appears to become slightly raised. The onset of progressive relaxation of the ligaments coincides with the onset of softening and dilation of the cervix. Complete relaxation of the posterior border of the pelvic ligaments, the so-called bands, is generally followed by delivery within 12 hours. Rupture of the first water bag heralds the onset of true labor. Following rupture of this membrane, there is a temporary weakening or cessation of straining, which resumes as the second water bag (amnion) enters the vulva [bob136.jpg].[1] The thick, slippery, slimy fluid contained in this bag provides lubrication for the delivery once it ruptures. The average interval between rupture of the first and the second water bag is about one hour.

Calving assistance

The minimum supplies needed to provide assistance at the time of calving are a ready supply of clean water, two buckets, soap, lubricant, two obstetrical chains plus handles, oxytocin, and seven percent tincture of iodine. When there has been no visible progress for two hours after the appearance of the membranes, the cow should be examined to determine the cause of the delay as well as the type of assistance she may need. Heifers are slower to dilate and should be given more time than cows; however, there should be evidence of progress. The calf will often live for eight to ten hours in the uterus after the beginning of true labor that begins with the rupture of the first water bag. The golden rules of obstetrics are CLEANLINESS and LUBRICATION. It takes from two to six hours for the cervix to completely dilate in the average cow, and from four to ten hours in the average heifer. The actual expulsion of the calf takes from one to four hours in the cow and from two to six hours in the heifer. The fetal membranes (afterbirth) are normally delivered in one to eight hours. They are considered retained if not delivered within 12 hours.

The plan

It is very important to not start to pull, by trial and error, on the first exposed part of the calf. A flowchart of the sequence of events[5] and what to do at each step is also presented in the Visual Guide to Reproduction, http://drostproject.org [bob298.jpg].[1] The internal examination is aimed at determining whether the calf is presented head first (cranial presentation) or tail first (caudal presentation) and whether the head and neck and both limbs are present and fully extended. At the same time it is decided whether or not the calf is alive.

Guidelines to determine if there is room

Cranial presentation (head first) [bob100.jpg].[1] The entire head resting on the knees and both feet must be presented in the birth canal. Chains are looped around each foot just below the dewclaws with the large link on top so the pull comes off the dorsal surface [bob139.jpg].[1] There will be sufficient room to pull the calf, if one person can pull the first leg until the pastern is 15 centimeters outside the vulva and, next while holding the first leg in this position, if again one person can pull the second leg equally far outside the vulva. At these distances both shoulders of the calf will have passed both iliac shafts, and the pelvic inlet. The diameter of the calf is greatest at the points of the shoulders.

Caudal presentation (backwards) [bob101.jpg].[1] Approximately five percent of the time calves are born backwards, and almost 50% of those lead to dystocia.[2] This presents two problems: 1) the blunt shaped hindquarters are less efficient in dilating the birth canal than the cone shaped head and neck, and 2) the umbilical cord becomes compressed against the pelvic inlet while the head is still inside the dam. Again, chains are looped around each foot below the dewclaws with the large link at the front of the foot so the pull comes off its dorsal surface. If, with the cow lying on her side, it is possible for two people to pull both hocks on a rotated (see below) calf far enough for the hocks to appear at the lips of the vulva, then there will be sufficient room to deliver the intact calf by way of the vagina.

Preparation of the cow for pulling the calf

While the cow is still standing, she should again be washed with soap and water, and the degree of dilation of the soft tissues of the birth canal should be evaluated. With folded fingers, both well-lubricated arms are inserted into the vulva and vagina like a wedge; next the tissues are stretched by pushing the elbows outward. Up to 20 minutes may be required in some heifers to fully dilate the vulva and the vulvo-vaginal sphincter. The preparation

will not only minimize tearing but it will also speed delivery once the process of extraction is started. Next the cow is cast; usually on her right side. She can be laid down by tying her head low to the ground to a post and by tying a long rope around her neck with a non-slip knot and then by placing two half hitches around her body. The first half hitch is placed tightly just behind the front legs, the second just in front of the hind legs and in front of the udder. By pulling on the free end of the rope straight behind the cow, she will be made to lie down and can then be rolled onto her right side. The advantages of laying her down are: 1) she can angle her pelvis more favorably by bringing her legs forward and she can slightly spread her legs, 2) the people pulling can sit on the ground and exert more pull, 3) the calf does not have to come up out of the abdomen against the force of gravity, and 4) she does not fall down in the middle of the extraction process.

Rotation of the calf

A cross section of the entrance into the bony pelvis (pelvic inlet) of the cow is shaped like that of an egg with the small end down. The vertical diameter of the pelvic inlet is greater than the horizontal diameter [bob163.jpg].[1] This means that the opening is taller than it is wide, and wider near the top than near the bottom. On cross section, the pelvis of the calf is wider at the hip joints (which are located below the hooks), than it is tall. The horizontal diameter of the fetal hips is greater than the vertical diameter [bob164.jpg].[1] Therefore, rotation of the calf allows its widest portion (the hips) to come through the greatest diameter of the pelvic inlet. However, the calf must be rotated before its hips engage the pelvic inlet [bob165.jpg].[1] The hip joints of the calf are sometimes too wide to pass horizontally through the pelvic inlet. To anticipate and prevent this, the hips should routinely be rotated to permit passage through the bony pelvic inlet [bob166.jpg].[1] The operator positions himself/herself on his/her knees next to the rear legs and udder of the cow [bob148.jpg].[1] For a calf in cranial presentation, rotation is started as soon as the head is outside the vulva. The operator passes his/her arm nearest the cow between the legs of the calf and above the neck. The other hand and arm are passed completely underneath the calf, and the fingers are locked near the base of the neck. The head can then be pulled toward the knees of the operator who rotates the calf while traction is applied.

When the calf is in caudal presentation, rotation must be started as soon as the operator has access to the legs, that is, before the fetal hips have entered the pelvic inlet. Again the cow is cast on her right side. Everything should be ready before the final pulling is started because once the umbilical cord is pinched the oxygen supply to the calf is shut off.

All pulling is done intermittently and only while the cow strains, upon command of the operator. This gives the cow, the calf, and the assistants brief periods of rest before the next maximum effort. The only exception to this rule is when the hips of a calf that is coming backwards, have just come through the vulva. These calves cannot breathe because the head is still in the uterus and their oxygen supply via the umbilical cord has been cut off. Continuous traction is applied until such a calf has been delivered.

Care of the calf immediately after delivery

Delayed passage through the birth canal in the face of a faltering placenta compromises oxygenation of the calf. Although the calf is able to breathe as soon as its nose passes the lips of the vulva, expansion of its chest is restricted by the narrow birth canal. This situation is made much worse when continuous forced traction is applied. As soon as the calf's head has passed the lips of the vulva, traction should be interrupted, the nostrils cleared of mucus, and cold water applied to its head. When the calf is completely delivered, immediate attention is directed toward establishing respiration. Mucus and fetal fluids should be expressed from the nose and mouth by external pressure of the thumbs along the bridge of the nose and the flat fingers underneath the jaws, sliding from the level of the eyes toward the muzzle. The common practice of suspending the calf by the hind legs to "clear the lungs" must be questioned [bob142.jpg].[1] Most of the fluids that drain from the mouth in these calves come from the stomach, and the weight of the intestines on the diaphragm makes expansion of the lungs difficult. The most effective way to clear the airways is by suction.

Respiration is stimulated by many factors, but only ventilation of the lungs, cooling and certain drugs allow us to render help immediately. The best stimulus for respiration is ventilation of the lungs. Sudden cooling is a very important respiratory stimulus that can be elicited by simply pouring cold water over the head of the calf. Cold water elicits the gasp reflex that aids in the expansion of the lungs. Brisk rubbing of the skin or tickling inside the nostril with a piece of straw also has a favorable effect.

Accidents of gestation/teratology

For the purposes of client education it is interesting to be able to illustrate a number of uncommon conditions to aid in the explanation of differential diagnoses. For example, the difference between hydrops allantois

and hydrops amnii. How the adventitious placentation of a cow with hydrallantois makes her unsuitable for rebreeding; how the profiles differ from one-another and from a cow with bloat. How a vaginal prolapse differs from a uterine prolapse in timing and appearance.

Teratology can be subdivided into fetal monsters and congenital anomalies. How does one explain a *Schistosomus reflexus*, or a globosus amorphus? A picture is worth a 1,000 words in those cases.

Turn to reference 1 and select the Subjects of Accidents of Gestation and Teratology.

Advanced reproductive technology

The category of Advanced Reproductive Technology is a slightly esoteric for the purpose of training farm personnel. Yet, images may help explain the process of embryo flushing, and illustrate the developmental stages of the early embryo from day to day (Subject Reproductive Technology).[1]

References

1. Visual Guide to Reproduction (http://drostproject.org). Select: Bovine Reproduction Guide.
2. Roberts SJ: Veterinary obstetrics and genital diseases (theriogenology). 3rd ed. Woodstock (VT): Published by the author; 1986; p. 283.
3. Des Coteaux L, Colloton J, Gnemmi G, edtors: Practical atlas of ruminant and camelid reproductive ultrasonography.Ames (IA): Wiley-Blackwell; 2010.
4. Lucy M, Green J, Poock S: Chemical pregnancy tests in the bovine. Proc Dairy Cattle Repro Conf. St Paul MN; 2009. p. 29-40.
5. Schuijt G, Ball L: Delivery by forced extraction and other aspects of bovine obstetrics. In: Morrow DA, editor. Current therapy in theriogenology. Philadelphia: WB Saunders; 1980. p. 251.

Images

Illustrations of the various procedures, instruments, and anatomical specimens may be viewed in the Bovine Reproduction Guide under the respective Subjects. To view an individual image, select Search on the Home Page and enter the file name (e.g. bob136.jpg. Alternatively, the QuickSearch box in the lower right hand corner of each images can serve the same purpose.

Marketing of the food animal reproductive practice

John Myers
Vinita, OK

I've always been impressed with the quality and depth of the science presented at the Society for Theriogenology (SFT) meetings and I am proud and indebted to those scholars who have come to these seminars and offered detailed and voluminous data from which they make logical, if not limited findings. I am, however, not one of those scholars. It is my endeavor to explain how I market myself as an exclusive food animal practitioner and, as is my custom, not be compelled to justify my conclusions by any sort of rational details that might back them up, or recommend anything for any other reason that it seemed to me to be a good idea at the time.

First, a few ground rules. You will not learn specifics about how much money I make. I feel comfortable with this because I cleared it with Dr. Jane Barber who said she did not reveal that statistic for herself when she did a marketing talk on a small animal reproductive practice. The statistic is important, however, because it represents a number by which many of you will judge whether I am a successful practitioner. The honest answer to how much I make is the same answer each of you gives when asked how much you make, and that is "not enough". Also, the honest answer to how much you need to make is the same for you and me also, and that is "more".

When working on this presentation, several truths or verities were driven home to me.

First, without exception, the following to truths arise so often they must be true:

Everyone wanted to be a large animal practitioner–or,

Everyone's favorite teacher in veterinary school taught large animal medicine.

Second, this next fact is usually brought up sometime between when I'm in the middle of delivering an emphysematous calf and the moment I figure the bill:

Nobody can make money in large animal medicine.

Now this statement has a corollary that my children hammer home to me since they've moved to the big city and have to pay for veterinary care for the dogs:

All the money is in small animals.

This eventually leads to the truest truism of all:

Nobody makes any money in veterinary medicine.

To outline what I'll cover today, here are the major points:

- My background and locale
- Major components of my practice
  - Breeding soundness examination (BSE) of bulls
  - Female: artificial insemination (AI), embryo transfer (ET), ultrasonic (US), pregnancy diagnosis, fetal sexing by US
  - Lameness and foot trouble
  - Regulatory and organizational
  - Laboratory
- Advantages to my practice
- Disadvantages
- Application of theriogenology (the term and board certification)
- The experiences with student externs
- The food animal practitioner shortage

I stated that I would not be bound by anything scientific to make my points, and neither will I be bound by any sort of definition of marketing that would conform to how the term is actually used or defined. For the purposes of this discussion, I choose to define marketing as presenting oneself for personal gain.

It struck me that with that definition virtually everyone is in the marketing business whether one is a large animal practitioner, a dean of a veterinary school, a horse trainer or a hermit. We each make conscious and unconscious decisions about how to dress, how to express ourselves, what kind of car to drive and how often we think the lawn should be mowed.

It furthermore struck me that these unconscious decisions may be equally as important as the more deliberate and conscious ones. You know that the striped slacks have a slimming effect but you keep shoving them to the back of the closet because the khakis are a little looser around the waist and you are more comfortable. It's not that you don't want to look slim, but one day you notice your entire wardrobe is composed of khaki slacks and sweatshirts. Later we will address this comfort issue.

Another aspect in the definition must involve to whom one presents himself or herself to gain personally. Now I have nothing against Baptist preachers. Let me be very clear about that. I come from a long line of them; my

favorite uncle–the one who performed the wedding ceremony for my wife and me–is a Baptist preacher. As a large animal practitioner, however, while I certainly would not intentionally offend or insult such men of the cloth, I am much more concerned about my presentation to beef cattle owners than I am, for instance, to Baptist ministers. So in the marketing discussion I need to define to whom it is that I present myself. I must therefore tell you a little about my state and my portion of the state.

Oklahoma is a Choctaw word that means "Home of the Red Man". We're a young state, just over a hundred years old and only Arizona, Hawaii, Alaska and New Mexico were admitted into the union after we were. The parts of the states that were not dedicated to Native American reservations were settled by land runs whereby settlers would gather at a specific point on a specific date and at the sound of a gun would dash off on horseback and stake claims around the land they wished to own. Obviously some cheated. Instead of waiting at the starting point with God-fearing, hard-working, industrious and civil people some despicable types would invade the land before the gun sounded. They went in too soon. These people were called "Sooners" and they are as hated to this day by the same God-fearing citizens as they were in the late 1800's.

So as a young state, our culture does not have to look back very far to see that its ancestors were valued for having fast horses, good livestock, a hardy disposition, intent on hard work, and the ability to judge land. We have produced no great statesmen, no superior literary figures, and very few artists of international acclaim. As a state we have major problems funding education, keeping our population healthy and dodging tornados. The population of Oklahoma represents approximately one percent of the total US population and we have, to our credit, produced a disproportionate share of people with physical attributes such as major league baseball players (6) and Miss America winners (6).

The northeast part of the state, where I practice is the home of one of Dr. Peter Chenoweth's favorite Americans, Will Rogers. Will was born in Oolagah, in Indian Territory and went on to be a performer in the Ziegfeld Follies as a trick roper, then continued in the public eye as a newspaper columnist and movie star. He was quite a benevolent person and is Oklahoma's favorite son having his image in the Capitol in the hall of statues. It was common a few years back to have the name "Rogers" appear on more than 50% of the names on a statewide ballot, and most people in northeast Oklahoma can trace ancestry back to him. I can do the same.

He stands in another room but the other Oklahoman with a statue in the Capitol is Sequoyah, the developer of the Cherokee alphabet. Sequoyah's statue was sculpted by Vinnie Ream, who in addition to that work of art also did Abraham Lincoln's statue in the rotunda of the United States' capitol, and has a town in Oklahoma named after her. That town is Vinita, the county seat of Craig County and the location of my practice and the birthplace of my three children.

It is here in Craig County that I practice, in a county with a population of around 15,000 and about three times as many beef cows as people. Although there are a few stocker operators, the main agricultural industry is beef cows with several seed stock producers and commercial cowmen. While most of my clients are old, white males, with few exceptions the same family has owned most of the ranches since statehood, even though there is now a perceptible shift in ownership to larger landowners whose primary occupation may not be in agriculture. I estimate, however, that ninety percent of my business is from private owners and not corporations and basically these clients are primarily professional farmers or ranchers or in the very least their investment in their land is such that their agricultural pursuits would never be considered as a hobby.

So how do I present myself to these older, white, conservative males who live very modestly, read voraciously, and to say the least are very frugal? Or, how do I present myself for personal gain?

We need to go back to the khakis in the closet. When I arrived in Craig County, fresh out of the Army in 1973 I was full of ideas that I had been taught in veterinary school that would rocket me to stardom in the veterinary world and, coincidentally, turn all the ranches in my practice area into money making machines. I guess I also wanted my pants to have a slimming effect. Looking in the closet, however, I see comfortable clothes, and the fashionable wardrobe–like most of the ideas I had about what I would do with my practice–have been pushed to the back, never to be worn or used again.

So what has worked? In 1997 an idea I heard at a SFT Conference was executed in Craig County by our County Agent, Roy Ball and me. Roy had just moved to Vinita and is aggressive, hard-working, and opinionated and received in varying degrees by the people he serves. We had a pharmaceutical company, Hoerst-Roussel at the time, then Intervet, then Intervet/Schering Plough, now who knows what the name of it is, donate fenbendazole wormer for what we thought would be about 25 bulls. We reduced the price of our BSE, gave a leptospira bacterin and de-wormed the bull on a certain day in April, about two to four weeks before our spring breeding season.

Roy put out some brochures, I wrote a letter to my clients–I'm guessing about 200 of them–and we scheduled a producer meeting in the week of the bull clinic. The meeting we called "Cowboy College"–Roy's term and not mine–and at the end of the meeting we had a test over the material covered and awarded prizes to the high

scoring individuals. Intervet, or whoever they were at the time provided a meal and participated in the program and the prizes.

About 150 people showed up for the meal and later in the week we tested about 40 bulls, so we considered it a success. The concept of the Cowboy College needs further examination. Of the attributes I listed of the cattle producers in our area, I did not mention competitiveness. The winners strutted around the fairgrounds building as if their cattle had topped the market, and the losers came up afterward and bitched about trick questions.

The discount for the BSE's, even including the free vaccination and de-worming, still did not make the services to my clients in line or cheaper than my competitors. I foolishly thought, because I had been brought up with the SFT's guidelines on examinations, I needed to measure and palpate the testicles, perform a rectal examination to include seminal vesicles and inguinal rings, a visualization of the entire penis, and a motility and morphology examination of the semen. While I think I'm fairly efficient, I can do about ten an hour, and at the moment I charge 45 dollars for that service.

My competition, however, has a helper insert (ram might be a better word) a rectal probe in a bull's back end, and catch the first drop of something that looks interesting coming out of the prepuce and look for motility. I said "motility", but the scientific term is actually "swimmers". The typical charge for that service is somewhere between$12.50 and $20.00 and there may or may not be any sort of certificate supplied at the time. In effect, the people against whom I'm competing could have charged for the wormer and vaccinations and performed their service and beaten our discounted price by fifteen to twenty dollars (Table 1).

We continued on, however, and the bull clinic has grown, as has the total number of BSE's I do throughout the year. I perform about a thousand BSE's, it brings in more money than any other service I provide, and it leads to virtually every other service I perform. While this may not work as well in other practices, doing the best job on this examination from a marketing standpoint is either very logical or, if I may say it, brilliant.

I envy my wife as a caterer. People come to her and they are always happy. They are having a wedding, or a reunion, or a baby shower or a fiftieth wedding anniversary. Happy times. I walk in and I've diagnosed lymphsarcoma in a client's donor cow.

Except on BSE's. There is always good news, no matter what. If the bull is deemed fertile, life is wonderful. If the bull has problems, it's always good that we found out beforehand. The Society's BSE is a marketing tool. My clients now are accustomed to the form and know how to read it, and if they buy a bull that was examined by someone other than me, they have become suspicious if they do not receive some sort of document, preferably the SFT form, as part of their papers. Finally, never imagine me as a careful and thorough individual, intent on details. My clients, however, expect care and time to be taken by me in the examination of their bull. If I'm tired or out of sorts for one reason or another, I can always sense from the client that I must take my time and be as particular as possible even if it's the end of the day or my son is about to play basketball.

This, however, is a khakis in the closet discovery, not a calculated marketing scheme that I designed to bring me personal gain, although it certainly does. I provide a service that my clients and I enjoy and appreciate so it becomes comfortable unlike other schemes such as newsletters, web pages, bigger telephone directory advertisements and signs at our entrance. I've tried all of these things and they do not seem to appeal to my clients although they might impress, well, some Baptist ministers.

Table 1. Comparison of "Breeding Soundness Examinations" offered by practices in the area.

	Pecan Drive	Clinic A	Clinic B	Clinic C
Rectal	Yes	Yes?	No	No
Scrotal Measurement	Yes	Yes	Yes	No
Entire Penis Visualized	Yes	Yes?	No	No
Motility Examination	Yes	Yes	Yes	Yes
Morphology Examination	Yes	Yes	No	No
Cell Counter	Yes	No	No	No
Theriogenology Form	Yes	No	No	No
Number/Year	1022	50	125	35
Cost to Client	$45	$20	$30	$27.5

This one service has spawned other services. In our area the abundance of fescue pastures, inappropriate nutrition, suspicious genetics and abundant rainfall result in foot pathology, especially in bulls. Several years ago I attended a seminar at the American Association of Bovine Practitioners (AABP) meeting in Nashville put on by Jan Shearer and finally got a handle on how to trim feet. Consequently our practice does a lot of foot work and it's the hardest work I do. It is hard for two reasons. One, even with power equipment the work is very physical, and two, the hydraulic tilt table I have–while I get good exposure to the lower limbs–does not restrain the animals as well as I would like. If you have an answer to a better chute, I'd appreciate knowing about it. Nevertheless, we bring in clients from 150 miles away to work on feet. I try to encourage many of these people to take their bulls to the veterinary school in Stillwater, and I've tried to run them away with higher prices and a sorry attitude but it's not unusual to pull up to the clinic in the morning and have ten fat and angry Angus bulls needing foot trims. My typical charge for this service is just over $100.00

On the female side of the clinic's services, I do a fair amount of pregnancy examinations, usually in groups of 100 to 500 at a time and I probably do a little over 10,000 females a year. I have no idea how I compare in price, accuracy and congeniality with others who provide this service, but it takes a clever receptionist to get five to six thousand cows checked in groups averaging 250 when virtually everyone wants the work done on the same day. We also do a fair amount of ultrasound work, especially on heifers and females going into production sales.

We do many heifers 30 days after the bull has been removed from the herd so that marketing decisions on the open heifers can be made as soon as possible even though the cost for this service will be twice what checking them manually would be. Further, sex determination by ultrasound is expanding as one of our services. Once again, I honed my skills on this technique at a SFT symposium that took place in San Antonio.

I am involved in ET, although most of what I do is transfer ova. I have worked with several ET specialists, both veterinarians and non-DVM's, and while this work has tapered off significantly in my practice (attributable to finding genetic defects in my clients' herds) I have found that having someone else flush and freeze works out better for me than performing the entire procedure, which I used to do, with no other professional help. I learned the hard way that balancing a clinic schedule while trying to accommodate a flushing schedule exceeded my capabilities. I had to decide if I wanted to be an ET specialist–which would probably require more travel than I care for–or be a clinician. I chose the latter.

Our clinic is also involved in a small amount of AI. We will synchronize and breed an occasional group of 100-200 heifers and from time-to-time we will house and breed small groups of 10-15 cows at our clinic. We usually house, superovulate and breed all the donor cows undergoing ET at our clinic. As a note of interest, we have in the past been contracted to AI a group of cows owned by a corporation while other cows owned by the same people have been inseminated by experienced non-DVM technicians on other parts of the ranch. With numbers adequate to satisfy my own statistically challenged mentality, I've found veterinarians, young and old, male and female, experienced or not in AI can usually match or more probably exceed the results of non-veterinary inseminators. This has surprised me because it seems like nobody knows more about bovine reproduction than someone who has attended a two-day AI school.

A question arises about my method of charging–whether by the hour or the task. I charge by the task almost exclusively. I envy the dairy practitioner who, by virtue of doing most of his work in a barn, can work all year round. My practice has two distinct humps with the peaks generally in April and October. In the heat of the summer and the cold of the winter I simply don't have much work, and to make up for this I must make my money when I can. While I'm working I can make around 300 to 500 dollars an hour, and while my clients recognize this, my rates would be around twice what the most arrogant attorney in our town charges.

The remainder of my practice consists of examination and treatment of the individual animal, some regulatory work–I inspect a feed, water and rest station for the USDA for dairy cattle shipped from Canada to Mexico–dystocias and practice management. I've got three people that work for me, including my wife on a part time-basis, and we get along well and as a group we are liked and enjoyed by clients.

A word about staff meetings: we have them. They are conducted in the pickup on the way to work cattle. I understand the importance of personnel management but suggestions I've read about how to conduct that have left me confused. As with web pages and newsletters so it is with staff meetings. Somebody has to take responsibility to get things done. The manager of our electric cooperative said that in his evaluation his senior staff always wants more staff meetings. Improve communication, develop bonds, establish priorities, etc. So it is at our clinic. The problem both for the cooperative manager and me is that we (he and I) must organize, set the time, meeting location and agenda, hand out responsibilities and have a method to check on results. It's tough for him to delegate those items with a far greater staff than I have and so we've both found the first staff meeting works great while each succeeding one loses its punch. After a while the meetings don't happen until someone complains that the reason for our troubles is we don't have staff meetings.

I mentioned web pages, and I have some experience with them. I don't know whether a catering business or a large animal veterinary clinic would benefit more from a web page. Several times my wife and I are asked about the ability for someone to find information on our individual businesses. My wife got many more inquiries than I did so she had a web page developed by AT and T. In one year she got 100 hits and virtually no business directly related to the web page that cost her $80.00 a month.

I do know, however, that my son is what is called a search engine optimizer. It's his business that when certain words (in this case insurance) are put into a Google or whatever search engine that his company is at the top of the list on the first page of the search. He wanted to do the same thing for my clinic a few years ago. I was afraid that I would either get a number of phone calls just wanting information that would not lead to income, or that I would get more business, probably foot trimming, that I might be reluctant to handle. I do, however, have an approved USDA laboratory where I perform tests for equine infectious anemia (EIA), so I let my son optimize my laboratory but not my clinic. Within a week I was number two on the search engine list when you put in "Coggins", "veterinary laboratories", "EIA testing" and the like. It resulted in business and good contacts and exposure until a conflict at work required my son to abandon the project. So my experience with web pages has prompted a feeling of beware of what you wish for.

I am an exclusive bovine practitioner, and I believe that exclusivity leads to several advantages and corresponding disadvantages.

- Efficiency
  - Drug inventory
  - Equipment and facility
  - Education
  - Reputation
  - Time management of appointments
- Predictability
  - Boredom from routine
  - Feast or famine
- Physical
  - Healthy? Physically fit?
  - Fatigue, wear and tear, exhaustion
  - No appreciable after hours call
- Cyclical
  - Have definite down times
  - With cyclicity comes expanding and contracting bank account
- No dispensing
  - Questionable profitability
  - An ethical problem
  - Removal from management
  - No free clothing
  - No detailing
  - No appreciation of influence

I've got three more areas of conversation. The first is about theriogenology, the term, the college, and benefits. While I won't go into it in detail, my quest for board certification was not a thing of beauty, and once I received it there was, for me and others, a period of confusion. My close friends congratulating me on being certified in whatever it was, some colleagues were impressed and other older ones had never heard of the discipline, one client wondered if I accomplished this because my conception rate on ET had improved and customer of another veterinarian in town brought me an international health certificate to Canada to be endorsed. If I am honest with myself, this certification brought me great personal and professional satisfaction but the stars had to align perfectly for me to fit the study required in between the birth of our third child and the commencement of following my oldest two children in their athletic careers once they entered junior high. I think the only people in town who knew and understood the term "theriogenology" were my wife and children and the employees at the clinic.

I will say, however, there are close to three hundred of my clients who can read and understand the SFT Bull BSE form. Also, no less than fifty veterinarians have asked me about board certification and while they are unwilling to devote the time to learning as much as what is currently asked, they are acutely interested in some sort of partial recognition or certification in the particular specialty they pursue. I understand this is probably beyond the capabilities, policies and legal precepts of the college and society. However, had I first encountered BSE's as done

by veterinarians who just look for "swimmers" I might have missed out on some financial success and my clients would have suffered from an inferior service. I'm in favor of spreading competence that exists in theriogenology as far as possible, and if a partial sort of recognition helps that along so much the better.

Secondly, I would like to visit about how student externs are an important part of our marketing of Pecan Drive Veterinary Services. Our fortieth student left our clinic in May; thirty-nine of them have been absolute delights. Without fail these young men, and mostly young women, are enthusiastic, polite, inquisitive, hard-working and attractive. The only thing that brings them more joy than having a nerve-wracking busy day at the clinic is to get a call at midnight of that same day with a cow that's been in labor for five days.

We take them to church, Lion's club, trail rides and fish fries. They will eat lunch with about twenty-five to thirty ranch hands, and one young man spent time after clinic hours helping build a playground for our elementary school. Our clients are accustomed to them and are more than willing to let them do whatever I think will not cause any harm, and our students and clients remember one another long after they've gone back to school. I'm a great fan of the St. Louis Cardinal baseball player Matt Holliday. If to promote and market my clinic I had the choice of having Matt ride in the pickup and say good things about me or a veterinary student interested in food animal medicine and squirming to get to the ranch and get a face full of cow manure it wouldn't even be close. I'm so high on what I've seen come out of the 13 veterinary schools that have trusted us with their students that I think the profession is in very good hands.

Finally, I'd like to talk about the food animal practitioner shortage. I really cannot make intelligent conversation about why this is happening or what to do about it. What I can tell you is how it affects me and how this all fits into a marketing discussion. At this stage of my career, I'm not interested in working much harder than I already do. Because of that situation, I have enough business to live the life I desire, but not enough to hire someone else. I've been in group practices all of my life, and in previous presentations at the Oklahoma State Veterinary School I've encouraged students to look for multi-practitioner practices. Being by myself, however, I find I'm working less, enjoying life more and making more money than I ever have.

The marketing concept prevalent at the time I entered practice, however, was to work hard until your knees, back or heart gave out, and then sell your practice to some young veterinarian. The purchase price of the practice was your retirement. I was late in recognizing that I might not have a practice to sell, or put differently, I might not have a practice anyone would be interested in buying. I don't think I'm alone in this respect.

I find it interesting how many children follow their parents in the same profession, and indeed there are numerous examples in Oklahoma where sons and daughters of veterinarians have become veterinarians themselves just like baseball greats Bobby Bonds and Ken Griffey spawned Barry and Ken, Jr. None of my three children have shown any interest in following either my professional footsteps or those of my wife as a caterer. Further, all of my children work for large corporations and not for themselves. While I am not disappointed in this at all, I do wonder if they did not see the rewards and satisfactions in our professions, or if they did were they payoffs insufficient for the work my wife and I put in.

With that track record, how do I market my practice to someone when those most familiar with it have declared it lacking? The answer, I believe, lies both with me individually and our profession of food animal practitioners collectively, and is the subject of this presentation and I assume the reason I was asked to speak. I think this once again goes back to khakis in the closet.

As I look back on my practice, I realize that my career has been a 40-year process of my clients and me adapting to one another. Marketing pre-conditioned calves is an example. That concept never worked for me no matter how aggressively I pursued it. The work I do in marketing bred heifers fell in my lap. One of the points of my speech is that I think there are existing aspects in every food animal practice that are already valued and appreciated but under-marketed. If BSE's suddenly fell out of favor I think I would join with a few clients and sonogram some heifers or cows for fetal sex determination, or buy some older but still worthwhile embryos from clients who no longer want that particular genetics and implant perhaps 10 or 12 for clients who would not otherwise consider it. I enjoy working with dehydrated calves. Perhaps I would restructure my charges on that service–a guarantee perhaps; someone suggested a partnership. While I know there are pitfalls in each of these ideas, the experience would nonetheless logically spread further. These would be comfortable for my clients and me. Comfort, however, can only go so far. While my clientele is my clinic's most valuable asset, I sense that my clients' comfort is more palpable with lower prices.

Individually I must do several things. I must pursue those avenues available to me that provide client and personal value and satisfaction, and abandon those activities that result in frustration and fatigue. I must arrange my professional life so that I have a personal life. I must raise my fees. If the statistics I've shown you exhibit nothing else, they at least state that my business–our business–is not based on being the low bidder on every project. I wonder how much easier it would be to attract young veterinarians into our specialty if we could offer them $50,000

a year more income than others aspects of practice with the same number of hours worked each week? If what we do cannot provide those sorts of compensations, it is a fault of marketing by food animal practitioners or there is not enough value in what we do to justify our existence. If that is the case, we deserve to diminish and die.

Collectively, we as a profession must provide support, recognition and innovation to each other and our upcoming colleagues. With communication capabilities that we now possess it should be easy, but in my case it is very intimidating. The AABP, the SFT and the American College of Theriogenologists all have listserves and I read some or all of them every day. I rarely respond or enter the conversation. What I would enjoy would be a small group of, say, ten primarily food animal practitioners–perhaps from different parts of the country–who would participate in discussions about our practices, our frustrations, our solutions and our futures. I would even like an academic or two involved in the group. Nothing pleases me more in a professional sense than when a fellow veterinarian commends the job I've done. Since there are so few food animal practitioners, and our numbers are rapidly dwindling, it should be easier to mobilize a few people to develop methods of recognition, support and mentorship.

I have not touched on many political implications in this presentation. I have not asked our associations or their lobbyists to carry messages to the state or federal authorities pleading the case for the kind of practice in which I'm involved. I am not prescient enough to predict what will happen five, ten or twenty years down the road. While it may seem self-serving, I'm not sure how I will be replaced in my community. Clients have expressed this concern. What I do know is I'm confident that if we communicate well to our clients and ourselves, we should be able to rejuvenate our section of the profession. As a small animal practitioner in a crowded and competitive area of Tulsa asked me when I told him of the food animal veterinarian shortage: "What part of being a monopoly bothers you?"

A final statement to my food animal practitioners involved in reproduction as a parting shot in this marketing presentation: We're small, we're valuable and we're powerful. We just need to act like it.

The science of political science

John Myers
Vinita, OK

Steve McQueen has always been one of my favorite actors and I considered him a giant in the film industry. In 1972 he and Allie McGraw starred in a movie entitled "The Getaway" which had as one of its characters an actor named Jack Dodson who was probably most known for his work on the Andy Griffith Show. In the movie Jack Dodson played a veterinarian married to Sally Struthers, an actress known for being Archie Bunker's daughter and the possessor of a great set of acting credits.

As part of the plot, a wounded outlaw, Al Letierri, kidnaps the veterinarian and his wife in order to gain medical treatment and–to the dismay of the veterinarian–special non-medical attention from his wife. The veterinarian is made to witness this attention and subsequently commits suicide.

In a veterinary journal published at the time an editorial appeared decrying the use of veterinarians in such an ungraceful manner. "If portrayals such as this continue," the editorialist said, "the image of our profession will be irreparably damaged." The article went on to suggest our profession do what it could to prevent such portrayals and at the very least recommend that we as a profession boycott the film. As if a concerted effort from the veterinary profession could successfully do battle with Steve McQueen.

I've often thought back on this editorial and noted how differently we address our image and public scrutiny now as opposed to forty or fifty years ago. I acknowledge other professions have gone through equally radical changes in that length of time, but at a time when ethical considerations precluded oversized telephone directory ads, pictures of animals on letterheads, and a dress code requiring students to wear neckties, I suppose it was not out of character for members of our profession to think that as a group with reputations as pristine as ours we could dictate to Hollywood who it could choose as villains and who it could not.

I think that within our profession a segment still exists that thinks we should be impermeable to change inflicted upon us by people who are not members of our vocation. Veterinary school is arduous, we work under demanding conditions, and anyone who thinks that we should behave in ways different from how we already do is suffering from a deficiency of understanding of what we've been through and how difficult our job already is.

The difficulty of this position explodes when we come in contact not with some entity that can stand on the outside and hurl insults or taunts, but with a group that can penetrate the fortress and actually change what we do. The group that can do this is our state and national legislators, and it is my intention to give three case studies that I think illuminates what I think we're up against.

As I've said before, I've never let accepted facts or definitions get in the way of what I'd like for my opinion to be, so I'd like to approach–in my limited understanding of science–the science of political science. As such, the definition I'll use for political science is "the study of the allocation and transfer of power in decision making."

Further, it has been stated that politics can be one of the five divisions of philosophy, the other four being aesthetics, logic, ethics and metaphysics. Politics for us, however, has a meaning not bound by anything other than raw emotion, gut feeling and deep-seated empiric prejudice. Our own common sense couldn't be clearer about the wisdom or foolishness in the process of decision making, and our emotions and confirmations rise and fall depending upon how closely the decisions directly affect us.

I also offer three definitions or descriptions about politics that I've gained while preparing this presentation. Two are from Oklahoma legislators and one is from an established economist.

1. "Politics is about getting just one little thing." Joe Eddins, Oklahoma House of Representatives, District 6.
2. "Politics is limiting freedom for one group for the betterment of another." Jon Ford, Oklahoma Senate, District 8.
3. "Politics is not the art of the possible. It consists in choosing between the disastrous and the unpalatable." John Kenneth Galbraith, Canadian-American economist

Case study number one: House bill number 1812

In 2007 a bill was introduced on the floor of the Oklahoma House of Representatives by a Mr. Don Armes, of Faxon, Oklahoma. With the backing of the Oklahoma Cattleman's Association and the Oklahoma Farm Bureau Mr. Armes' bill provided the ability for cattlemen to acquire prescription drugs without the necessity of a valid veterinarian-client-patient relationship (VCPR). This was not the first time the issue had arisen, and in preceding attempts the efforts had been repelled by work from the Oklahoma State Board of Veterinary Medical Examiners, the Oklahoma Veterinary Medical Association (OVMA), and the lobbyist for the OVMA, Otie Ann Fried. This effort, however, seemed better organized.

The Oklahoma Representative from my district, Joe Eddins–a Democrat, came as he always did every two weeks or so to visit about issues in agriculture, education and rarely, veterinary medicine. Joe and I were acquaintances–in our small town everyone is an acquaintance until the relationship graduates to friendship–until his youngest son, Tom, entered my Sunday School class.

Tom was an exceptional student, athlete and leader and brought with him to the class many of his equally bright and engaging classmates regardless if they were Methodists or not. While I made a halfhearted attempt at religion, I found the students in general and Tom in particular had a wide range of interests. I brought microscopes to the church and we looked at organisms in puddles; we had contests to name Nobel Prize winners and major league batting champions. Tom was the quarterback of the football team, a center on the basketball team, district champion in the high and low hurdles, worked for me and I helped in write his valedictory speech. His father Joe was appreciative.

So the visits Representative Eddins and I had delved more into the philosophy of politics and the inner workings of the legislative body than it did into any particular bill of legislation. It was Joe who introduced me to the concept of "one little thing." It was his belief that all conservatives want the government out of their business, and out of all businesses for that matter, and all they want is "one little thing." The trucking executive decries taxation and regulation except when there is an attempt to abolish permits he already possesses that prevent other concerns from hauling the same items, the Republican restaurateur wants nothing from the government until there is an effort to run a bypass around the city instead of by his café, and the wine manufacturer's representative whose contempt for any impediment to free trade wants no alteration in the antiquated Oklahoma liquor franchise laws that insures his position as the sole distributor of the companies he already represents. All of these feel that the teachers in our state make enough money and all they want from government is "just one little thing."

So one Friday afternoon in my office after work Joe asked me about the bill to relieve cattlemen from the requirement to possess a valid VCPR. His information was that the Cattlemen's Association and the Farm Bureau wanted just "one little thing." I explained to him the horror stories we all know about misuse of antibiotics in food animals and the public health concerns. He asked me if an official from the OVMA would write a letter explaining the points I brought up, put it on official OVMA stationery, and–most importantly–sign it.

On the day that the bill was to be heard, Joe ran copies of the letter and placed one on every representative's desk. He had done other homework with Representatives that he had helped with votes before; he enlisted the one MD and the one DVM in the House to help in the explanation of the human health issues. His experience along party lines–at the time there were more Democrats than Republicans (Joe is a Democrat, Don Armes is a Republican)–rounded up a few additional votes. When the legislators returned from lunch to vote on the bill, they found the signed letter from the OVMA on their desks. The Republicans immediately adjourned and went into caucus and upon their return withdrew the bill from consideration until an amendment was added that was satisfactory to the OVMA.

I'm not a particularly religious person, but I think one of the reasons for the veterinary victory on the house floor on this vote was that I taught Sunday School.

Case study number two

The study is about my modest venture into politics and it will be very brief. Last July, two days before the deadline for filing for the position, a friend of mine who is an employee of our local electrical cooperative asked me to run for trustee, or director, on the co-op's board. There was dissatisfaction with the co-op's management, and I was told I would have massive support from the employees. I would be one of nine directors and we represent around 38,000 members. I told the man I would run, but I would not campaign. Whoever he knew that would support me would have to do their best to get me elected while I sat on the sidelines awaiting coronation. After a day's thought on this strategy, however, I concluded this position was either the height of arrogance or cowardice and I dedicated myself learning as much as I could about the rural cooperative electrical business, the problems it faced and what issues were driving a campaign to get either me or my opponent elected.

I must now reveal a note about my personality. Despite appearances I consider myself a shy individual. I am reluctant to meet people and I have no proficiency in small talk. Further, I am not competitive; it's nice but not important if I win in poker or badminton. These characteristics may not be desirable when running for office.

During the campaign customers would ask me questions about what I planned to do if I were elected. Usually the questions fell in one of two categories–bizarre and slam-dunks. I learned to turn the bizarre questions into slam-dunks. The most beneficial slam-dunk was promoting common sense. Indeed, if elected I promised if

nothing else, I was going to use common sense regardless what my opponent–the incumbent–might choose to do. I found that I underwent a transformation. I wanted to meet people and I wanted them to ask me questions.

I came to several conclusions before and after the election.

1. I did not need to get all of the votes, just most of them. This allowed me the freedom to shrug off any negative comments I might have received that might have normally hurt me to the quick.
2. I rejoiced in all forms of support and did not differentiate between the size, wisdom or denomination of contribution. The person who shook my hand and told me she was genuinely glad I was running was appreciated just as much as the person who generously donated money to the campaign.
3. I found that I really, really, really did not want to lose. My opponent was a decent fellow but I had troubles with some of the people who were supporting him. His victory would validate some people's agenda with whom I had disagreements for several years.
4. Finally, after I was elected I discovered campaign promises are easier to make than they are to fulfill. I've spent a lot of time in board meetings, received a voluminous amount of training about the power industry and have done what I consider a great amount of private research to bring to the boardroom an intelligent and enlightened opinion. As you would expect, on easy problems the answers seem to drip with common sense. On difficult issues–use of coal, conservation, rates and environmental concerns–answers do not satisfy everyone, and whether the board in its decision making used common sense depends entirely upon which side of the question the observer stakes his claim.

Case study number three

"The Teeth Floaters Bill?"

Although all formal deliberations on this bill took place this year, the legislation to grant the ability to do equine dentistry began in 2009. In an effort to abbreviate the story and compress the complexity into manageable portions I'll introduce the players in the drama.

1. Don Armes, House Republican
2. Brian Renegar, DVM, House Democrat; Lee Denney, DVM, House Republican; Phil Richardson, DVM, House Republican
3. Jon Ford, Senate Republican
4. Oklahoma State Board of Veterinary Medical Examiners
5. Oklahoma Veterinary Medical Association
6. OVMA Listserve (DVM)
7. Bobby Griswold, bareback rider
8. Clem McSpadden, Hall of Fame rodeo announcer
9. James McSpadden, OVMA lobbyist; Otie Ann Fried, former OVMA lobbyist
10. Joe Carter, DVM; Jeff Hammond, DVM; Tina Neel, DVM; Mike Johnston, DVM; veterinary activists
11. The Flaming Lips

Supposedly the story begins with the arrest of Bobby Griswold, a non-DVM teeth floater, who had received four cease and desist letters from the Oklahoma State Board of Veterinary Medical Examiners for practicing veterinary medicine without a license. In Oklahoma, practicing human medicine, human dentistry or veterinary medicine without a license is a felony, a concept pushed through the Oklahoma legislature without opposition by the Oklahoma Bureau of Narcotics to close a loophole in state law and give narcotics agents in the state one more method of prosecuting criminals in possession of drugs with or without intent to distribute. The various medical disciplines had different legislators to sponsor this felony legislation and the sponsor of the veterinary portion of the bill was Dr. Brian Renegar.

In March of 2009 Bobby Griswold was arrested, and he had in his possession 11 bottles of xylazine, detomidine and yohimbine. Because of his notoriety as a rodeo star and his popularity with clients, there was outrage by certain vocal people and the matter was taken up by Don Armes. The OVMA and its lobbyist, Otie Ann Fried began doing the work to protect the veterinarians in the state and the DVM Listserve was fraught with concerns from its members.

About this time the results were released of an unofficial vote in the state. In addition to our state song, tree, rock, etc., Oklahomans got to vote for their state rock song. The overwhelming winner was "Do You Realize" by the Oklahoma rock group, The Flaming Lips. At the ceremony leading up to the official declaration by the Oklahoma House and Senate to make the song our official rock song, the bass player of the group, Michael Ivins, showed up dressed in a T-shirt and sports jacket. Emblazoned on the shirt was a hammer and sickle and

subsequently certain members of the Oklahoma House of Representatives were outraged and refused to declare their song as the official rock song. Oklahoma may have the lowest payment for teachers in the nation, and we lead all states in incarceration of women, but we do what we can to keep communism from creeping into our life.

I, of course, wrote my veterinary congresspersons stating my indignation at their action on the song–a song, by the way, I had never heard. Dr. Renegar wrote back to say he, in fact, voted for the song, Dr. Richardson said he did not and thought it was a waste of time to discuss it, and Dr. Denney called me immediately to say she had gotten more calls on this one vote than she had all her other votes combined. Now what does the vote on Oklahoma's rock song have to do with a change in the veterinary practice act? Not much, actually, except that it was pretty much a party line vote with the Republicans in the majority, and it very clearly displays the mentality of our state legislative body. This is not a criticism. I've come to believe that the House and Senate have the courage of convictions to do what they think is correct.

The 2009 session ended with nothing other than a directive to study the teeth floating issue and work on legislation in 2010. Throughout the rest of the year after adjournment both sides prepared for the battle in the upcoming year.

Before the 2010 year began, the OVMA changed their lobbyist from Otie Ann Fried to the McSpadden group with James McSpadden officially in charge of the veterinary section. James would be the grandson of Clem McSpadden, former majority leader of the Senate, Cowboy Hall of Fame member by virtue of his long service as an announcer, a man possessing encyclopedic knowledge of rodeo performers past and present, and I'm sure a friend of Bobby Griswold.

Things heated up when Don Armes introduced House Bill 3202. As customary there was a certain amount of debate, formal and otherwise, but no specific date brought forth for a vote by the House. The OVMA pleaded for the membership to contact their legislators as it looked as though the vote–while still in our favor–might be close. Our veterinary legislators Denney and Renegar were working diligently to keep the bill from coming to a vote without at least having input into wording. The Listserve was inundated with opinions, declarations, advice and predictions. Technical opinions about the constitutionality, much less the advisability of such a bill came from many sources, but it appeared as those legislators most influenced by two groups, the Farm Bureau and the Oklahoma Cattleman's Association, were certainly leaning toward approval.

The OVMA called for a rally at the State Capitol. Over three hundred veterinarians, veterinary technicians and office help showed up on February ninth ready to, uh, do something. We met in the rotunda with James McSpadden, our lobbyist, and were instructed to go our representative and urge him to vote no on HB 3202. A fellow veterinarian and I had driven 200 miles to be told to contact my representative–a member of my church–with whom I'm already visited extensively and was assured was on the veterinarians' side. Not wanting to waste the whole day, I walked around to the Representative's individual offices to talk to anyone who would listen.

My experience was surprising. I found each of the Representatives polite, intelligent, articulate and engaging. They were forthright with their opinions. None were evasive and quite honestly all of them knew more about the bill and its ramifications than I did.

I spoke to my Senator, Jon Ford, who was on the floor. He told me he would probably vote in favor of the corresponding Senate bill, but told me (as almost all the legislators did) that this type of bill–called a scope of practice bill–is the most contentious legislation with which he deals. His opinion was that in giving "a little more freedom" to the teeth floaters everyone would benefit. Further he thought controls were within the legislation that would insure veterinarians would still have control of the necessary drugs.

We heard a few days later on the Listserve our impact had been phenomenal. Attempts to modify the bill were rebuffed, and meetings were scheduled then cancelled between the bill's sponsor and the OVMA. Finally, a meeting was at called at 9:00 at night and Representative Armes stated he had the votes to pass but would consider minor adjustments to the wording. Representative Armes refused to deal with the Oklahoma State Board of Veterinary Medical Examiners because he felt they did not deal in good faith. In years previous he stated that he and the Board would come to what was to be a final compromise only to have the Board want to insert "just one more little thing" the next day. Therefore Representative Armes dealt with the OVMA, which meant one legislator negotiated with as many Association Board members as could be located on short notice by conference calls. The wording finalized that evening never appeared in the legislation. Perhaps the strategy of having the meeting suddenly and at night was savvy statesmanship by Don Armes, or perhaps he'd seen the movie "The Getaway" and figured the veterinarians if given the chance would commit political suicide.

Regardless, the word was that Representative Armes and the veterinarians had come to a compromise; the bill passed the House in March and was sent to the Senate. According to several reports, hot button issues such as abortion and gun control were also on the table yet within the Capitol no issue was creating as much noise as the

teeth floater issue. Further reports suggested that the easiest way for the Senate to deal with the controversy was simply not to let it appear on the floor for debate.

This was when a full-page ad, sponsored by four veterinarians and not endorsed by any entity that would claim representation of the profession as a whole, appeared in the Daily Oklahoman. I'll go into the wisdom of this later, but shortly after this two events occurred.

The first is that the lobbying firm–specifically James McSpadden, our lobbyist–resigned. From the lobbyist's perspective the issue became too controversial, and more importantly began to alienate too many politicians that might be beneficial in other matters by other customers of theirs.

The second event was the Senate, having felt that their hands had been forced, took up debate on the issue. The Oklahoma veterinarians, now without lobbyist representation, continued a cumbersome process of working for their best interest through conference calls with ten to fifteen board members and a membership raging for settling for nothing less than complete and total victory–compromise was not in the vocabulary.

Two words became very important. "Consisting of" was left out of the final wording where it had been inserted by the veterinarians. Teeth floating was to be defined as animal husbandry. At this time embryo transfer and ultrasonography were also inserted and Don Armes admitted publicly that the reproductive issues were actually at the base of this issue and public support for Bobby Griswold and thirty other teeth-floaters was merely the horse designed to carry the load.

So in those procedures excluded from the practice act it now said that animal husbandry, teeth-floating, embryo transfer, ultrasonography…. etc. Animal husbandry is not defined by Oklahoma Statute, so the definition, according to the Attorney General's office would necessarily come from Webster's Dictionary. There animal husbandry is defined as "care of animals" so with the two words "consisting of" being deleted after "animal husbandry" it now leaves it open that all animal care is animal husbandry and not the property of veterinary medicine. Some think this rendered the practice act, and veterinary medicine in the State of Oklahoma dead in the water.

On March 31, 2010 the Senate passed the bill, without "consisting of" in the language and it was sent back to the House with an emergency amendment. The House passed the amended bill on April 8, 2010 by a vote of 58-37, and the Governor signed eleven days later.

The Listserve and people closely involved with the issue spewed venom at the Governor's signing stating he was an absolute coward refusing to do what was right. In this same session the Governor vetoed three abortion laws and one gun control law, two of which were overridden. I will leave it to you to decide if vetoes of these bills were acts of courage in Oklahoma.

Now comes the part where the scientists draw conclusions from the data presented. This precedes using the conclusions to execute a plan to elicit the desired results, implementing the plan and then measuring the results. At least that's what I think science is.

We were examining how in scientific terms power was transferred and allocated in decisions that were made. It seemed that having suffered from disastrous results in retrospect we would have welcomed the merely unpalatable.

I cannot emphasize too strongly how volatile and passionate the Oklahoma veterinary population was in this situation. The persistent comment was that our degree, our education, our value as professional is now worthless. This is a strong comment when one reduces to zero the efforts of a life's work. I wonder where competence comes into the picture. If I lacked my veterinary education, my continuing studies, my board certification, my license and degree would I be competent to do what I do? Would that be enough to satisfy the needs of my clients?

I made a "C" in my first anatomy course. That's always bothered me and I'm sensitive about it. My anatomical knowledge, however, is far superior to a non-DVM teeth floater, but what does it matter if he's a little hazy the location of the pre-femoral lymph node of a cat? He has been around horses all of his life and probably understands their behavior better than I do. Which pieces of knowledge will gain him the most?

So this leads to three questions:

1. Are we competent enough?
2. Is our competency specific enough?
3. Are we too competent?

I have clients that will not trust a non-DVM to perform a breeding soundness examination on their bull. However, I know many non-DVMs more qualified to do so and will come closer to a true evaluation than would a host of small animal veterinarians unfamiliar with the process. Likewise, I should not perform orthopedic surgery on dogs although by license I'm entitled to do so.

I wonder, therefore, why the veterinary population agonizes about those without our training competing with our competence. If it's merely a degree and a license that separates us we need to agonize. I realize veterinary schools and state associations worry every day about what it is that they provide that will best equip our profession to do the best job. At least I hope they worry every day, and I am in no way critical of the job they do nor do I have any suggestions of how to do the job better. I understand, however, the insecurity and paranoia of veterinarians when a state legislature can with impunity turn the state practice act on its head. My emotion witnessing this has not been that I wished for a better outcome by the legislature as much as I wished I'd paid more attention in anatomy class.

A few years ago we visited the Accademia Gallery in Florence, Italy. There we saw Michelangelo's statue of David. This figure stands over seventeen feet tall and among all the attributes poured upon this piece of art one certainly has to admit it is big. There are arguments whether the scene depicted in the sculpture was before or after the battle, but in the serenity Michelangelo was able to invoke on David's face we recognize what it is to work and prepare well, persevere with courage and battle giants. The message I took away from the museum was that the little guy is huge. The world rejoices when the underdog wins. It's almost Biblical.

I am sure David had done a bit of practice with his weapon before that day. I can see him spending some time selecting the very best stones and contemplating what vantage points would gain him an advantage. I have no doubt David was confident his weapon was in working order, and I am convinced the members of his tribe recognized his ability with a slingshot.

Both the story of David and the story of Oklahoma's 2010 legislative session and its effects on the veterinarians in our state–and perhaps other states–are stories of power. The story of how power was transferred or allocated and the stories of the decisions made surrounding the events are analogous and lead me to conclude the following:

Nobody feels sorry for Goliath. The veterinarians in Oklahoma felt overpowered by the enormity of the House, the Senate, the Cattlemen's Association and the Farm Bureau. The way the conflict was presented, however, was that the 2000 veterinarians in the state took the role of Goliath and David was a bronc rider from Geary, Oklahoma.

Second, the skills we possess may not be adequate in every war we wage. It is not necessary to tell a profession that deals in retained placentas and pyometras that some of the work we do may not have a pleasant odor, but who among us willingly will engage with those that purportedly smell as bad as a group of politicians? The time to connect with our representatives is not during a crisis, nor should the committee meetings to determine negotiation strategy be held forty-five minutes before a decision must be made.

Finally, the metaphor of David and Goliath finally falters in its comparison with our political struggles. Before the battle against the Philistines in the Valley of Elah, David was a shepherd–someone who cared for animals just as we do. After the battle David went on to other things, some good and some bad. Our mission, however, is to return to the flock. With this as our mission we must work and encourage anyone who helps in that effort. We must remember it is our goal to care for the ultimate little guys–those who cannot speak for themselves–and not to kick some giant in the ass.

Embryo evaluation and pregnancy outcomes following embryo transfer in cattle

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Abstract

Evaluation and selection of appropriate embryos for transfer into recipients is of vital importance to establishing pregnancy and producing a healthy calf. There are several methods currently utilized to evaluate embryos produced in vivo or in vitro including morphology, biopsy, differential staining, and metabolic assays. Visual assessment of embryos for stage of development and quality grade is the most widely used method in practice for evaluating embryos. Embryos of higher quality grades have been repeatedly shown to be associated with greater pregnancy rates after embryo transfer. However, morphological evaluation of embryos is not an ideal predictor of viability and developmental competence, as misclassification of embryo grades can result in variation in pregnancy rates of embryo transfer recipients. Additional diagnostic information may be gained from embryo biopsy and preimplantation genetic diagnosis. These technologies may be used to accurately determine embryo sex and to screen embryos for certain marker genes associated with important traits prior to embryo transfer. An increased number of genes associated with normal and abnormal development have been identified in bovine embryos during the past ten years. Future applications of marker gene technology to embryo selection should lead to more accurate predictions of embryo viability and developmental potential. In this paper we review the methods used for assessment of bovine embryos produced in vivo or in vitro as well as pregnancy rates and fetal development following transfer of these embryos.

Keywords: Cattle, embryo, embryo evaluation, fetus, pregnancy

Introduction

The establishment of pregnancy and delivery of a live calf are the ultimate goals of an embryo transfer program. A critical step in embryo transfer is the evaluation and selection of suitable embryos either recovered from donor cows or harvested from in vitro embryo production. Embryo selection is an important factor that contributes to pregnancy success following transfer of embryos produced in vivo or in vitro.

Currently, there are several methods that may be used to estimate an embryo's viability. These include morphology, biopsy, differential staining, and assays of embryo metabolism or respiration. The ideal assay of embryo viability should satisfy the following criteria: 1) objective measurement, 2) non-invasive, 3) ability to distinguish individual or multiple embryos at a time, 4) rapid, 5) technically simple and user-friendly, 6) affordable, 7) highly predictable and 8) repeatable in all culture conditions.[1,2]

Visual assessment of embryo morphology is a commonly used method for evaluation of bovine embryos prior to transfer. However, depending on the intended use of the embryos, additional procedures such as embryo biopsy may be warranted. Embryo biopsy and analysis of embryonic cells are technologies that allow for determination of genetic sex as well as identification of genes associated with embryonic development and metabolism as well as other economically important production and health traits. The purpose of this paper is to review the methods for evaluation of bovine embryos produced in vivo or in vitro as well as pregnancy results following transfer of these embryos.

Evaluation of morphology

Morphological evaluation of embryos using a stereomicroscope remains the most widely used criterion for predicting embryo viability in cattle. The assessment of embryo morphology involves assigning a stage of development and a quality grade to individual embryos based on several observable characteristics. This method has the advantages of being quick, non-invasive, and technically simple. In addition, it does not require extensive equipment. However, visual evaluation of embryos is rather subjective and does require training and experience in order to accurately assign the appropriate developmental stage and grade.

In practice, bovine embryos produced in vivo are usually recovered from cows or heifers on Days 6 through 8 after estrus and breeding, corresponding to the morula to hatched blastocyst stage of development. Alternatively, embryos may be produced in vitro. In vitro embryo production (IVP) involves retrieval of oocytes from the ovary of the donor cow using transvaginal ultrasound-guided ovum pick up or from ovaries collected at an abattoir. The recovered oocytes are then subjected to in vitro maturation, in vitro fertilization and in vitro culture for seven days. Selected embryos, produced either in vivo or in vitro, may be either transferred nonsurgically into recipients or frozen.

When evaluating embryos, perhaps the most important morphological characteristic to be considered is whether or not the embryo is at the appropriate stage of development relative to the time post-fertilization. Systems used for evaluating embryos produced in vivo or in vitro are based on deviations in morphology relative to embryo age and stage of development with age being based on days from estrus for in vivo derived embryos, or from insemination for in vitro derived embryos.

Frequently used parameters for assessing embryo morphology include shape, color, number of cells, compactness of the inner cell mass, number of extruded or degenerated cells, number and size of vesicles, and the quality of the zona pellucida.[3] Deviations in embryo morphology have been associated with lower pregnancy rates. These morphological parameters have been incorporated into embryo grading systems. During the past three decades, several different ordinal scales have been used for grading bovine embryos.[3-7] A widely used system for embryo evaluation is that recommended by the International Embryo Transfer Society (IETS).[7] The IETS system is based on formulated guidelines for assigning each embryo a numerical code based on morphological integrity. This system includes stage of development codes of 1 (unfertilized oocyte or 1-cell embryo) to 8 (expanding hatched blastocyst), and grade codes of 1 (excellent or good) to 4 (dead or degenerating). The grading system is summarized in Table 1. It is recommended that, unless otherwise agreed, only excellent/good quality (Grade 1 or Code 1; zona pellucida intact) embryos be used in international commerce.[7]

The proportion of recipients that become pregnant after transfer of fresh and frozen-thawed embryos has averaged 60% and 45% or greater, respectively.[4,8,9] Higher pregnancy rates are usually obtained when blastocysts are transferred before they begin to hatch from their protective barrier, the zona pellucida.[10] Wright had reported that higher pregnancy rates were obtained from the transfer of blastocysts (64 to 66%) compared to morulae (44 to 53%).[11] However, other investigators have found no significant difference between stage of embryo development at time of transfer and pregnancy rates of recipients (Table 2).

Multiple studies have demonstrated that there are significant differences in pregnancy rates across different morphological grades.[3-5,11-16] A retrospective analysis by Hasler of data from three commercial embryo transfer operations found that embryo grade was an important factor in pregnancy rate, as embryos of the highest grade were associated with significantly higher pregnancy rates than embryos of lower grades.[15] Similar results, summarized in Table 2, have been reported by other investigators.

Morphological evaluation is by no means a perfect predictor of pregnancy success. Lindner and Wright observed that some embryos with a "poor" grade produced a pregnancy, while other embryos of "good" quality failed to result in pregnancy.[3] Furthermore, visual evaluation of embryo quality is subjective, and thus may be subject to bias by the evaluator. A study of agreement on embryo stage of development and morphological grade among experienced evaluators concluded that good agreement exists when predicting stage or the extremes of embryo grade; however, evaluators had less agreement when selecting embryos of intermediate morphological quality.[17] These differences between experienced evaluators in selection of individual embryos for transfer into recipients resulted in considerable variation in expected pregnancy rates.[18]

Compared to embryos produced in vivo, bovine embryos produced in vitro can be more challenging to evaluate for embryo quality. Difficulties encountered when evaluating in vitro produced embryos are likely due to their unique developmental and morphological features. For example, Grisart, et al. observed a delay in development rate of presumptive zygotes cultured in vitro.[19] In general, the types of morphological defects seen with in vitro produced embryos are similar to those of embryos produced in vivo. However, compared to embryos produced in vivo, embryos produced in vitro have subtle differences in gross and ultrastructural morphology.[8,20] For example, morulae produced in vitro have less pronounced compaction, variable amounts of coalescence of individual blastomeres, a grainy cell mass, and less perivitelline space. Some embryologists have reported that it is more difficult to discern extruded cells in the early blastocyst stages due to a reduced perivitelline space.[8,10] While in vivo produced embryos tend to be light brown to brown in color and translucent at the blastocyst stage, in vitro produced embryos may appear darker.[3,21] The number of cells per embryo harvested from in vitro production systems, however, has been similar to that of embryos produced in vivo.[22] In vitro produced blastocysts of higher quality grades have more cells than blastocysts of lower grades, and embryos that take longer to reach the blastocyst stage are of poorer quality.[23] At the ultrastructural level, embryos from in vitro co-culture systems have smaller and fewer junctional complexes among the blastomeres, and blastomeres may contain more cytoplasmic vacuoles.[20]

When examining the ultrastructural morphometry of blastocysts, those produced in vitro demonstrated deviations in volume densities in cellular structures involved in metabolism and altered embryonic differentiation compared to those produced in vivo. Blastocysts produced in vitro displayed decreased volume density of mitochondria and nuclei, increased volume density of lipid, and increased proportional volume of vacuoles.[24] Similarly, compact morula produced in vitro had increased volume density of lipid and vacuoles, decreased

proportional volume of total mitochondria, and increased cytoplasmic-to-nuclear ratio compared to compact morula produced in vivo.[25]

The transfer of in vitro produced embryos or somatic cell nuclear transfer (cloned) embryos may result in abnormalities of fetuses, placentas and calves. In cattle and sheep, these abnormalities were initially recognized as oversize offspring and were referred to as Large Offspring Syndrome. In addition to large phenotypes, other problems have been reported including increased rates of early embryonic death and abortion, extended gestation lengths, abnormal placentas as well as increased rates of hydrallantois, congenital deformities, and perinatal death.[8,14,26-29] Fetuses and placentas from in vitro produced embryos may range from normal development to subtle abnormalities such as abnormal development of fetal skeletal muscle or placental blood vessels, to more obvious problems such as increased body weight. However, because all of these pregnancies do not result in fetal or placental overgrowth, the term Abnormal Offspring Syndrome (AOS) was proposed to more accurately denote the range of characteristics seen with this syndrome.[30]

Anomalies associated with AOS can range from moderate alterations with little apparent compromise to the fetus,[31] to more severe alterations including increased abortion, dystocia, and neonatal death.[28,32] Characteristics associated with AOS may be grouped into four types.[30] Briefly, Type I AOS represents early embryonic or conceptus death prior to completion of organogenesis (approximately Day 42 of gestation). Type II AOS includes abnormal development of the placental membranes and fetus and fetal death between completion of organ differentiation and full term (Day 42 to approximately Day 280 of gestation). Type III AOS represents a full-term fetus or placenta with severe developmental abnormalities and no evidence of compensatory response by the fetus/placenta. Newborn calves are often severely compromised with altered clinical, hematological, or biochemical parameters; death occurs at parturition or during the neonatal period. Type IV AOS includes a full term fetus or placenta with moderate abnormalities; however, the feto-placental unit compensates and adapts to the compromising genetic or physiological insults and survives. These calves may be normal or they may have clinical or biochemical abnormalities.

Abnormal Offspring Syndrome has not been consistently linked to single genes or a specific pathophysiology. The occurrence of AOS is influenced by in vitro embryo culture conditions,[33] levels of maternal nutrients,[34] and, in the case of cloning, the cell lines from which donor nuclei are derived.[28] Fetal and placental abnormalities seen with AOS are consistent with disruption of epigenetic reprogramming and the expression of imprinted genes as well as altered regulation of nonimprinted genes.[35]

Embryo biopsy

Embryo biopsy allows for more advanced genetic analysis, and it is currently the foundation for embryo sexing and preimplantation genetic diagnosis. This technique involves extracting one or more blastomeres from a preimplantation embryo through aspiration or microsection. However, there are some detrimental aspects associated with this procedure. For example, biopsied bovine embryos generally have slightly reduced pregnancy rates after transfer into recipients compared to the transfer of intact embryos. The biopsy of lower morphological quality embryos and freezing can further reduce pregnancy rates following embryo transfer.[36-38] In addition, the biopsy procedure can limit export of these embryos to some countries because penetration of the zona pellucida leaves the biopsied embryo vulnerable to infectious disease agents.

Embryo sexing

Producing calves of the desired sex is often of interest to clients, especially dairy farmers who want heifer calves for herd replacements. This may be accomplished prior to the transfer of embryos into recipients by analysis of blastomeres from an embryo biopsy. Alternatively, sexed embryos suitable for embryo transfer may be produced by insemination of superovulated females with sexed sperm or by IVF of oocytes with sexed sperm.[39,40]

There are several techniques available for sexing bovine embryos, including polymerase chain reaction (PCR) coupled with gel electrophoresis (electrophoretic PCR), nonelectrophoretic PCR, loop-mediated isothermal amplification, and amino acid profiling. Sex determination by electrophoretic PCR enables amplification of Y chromosome-specific sequences from a small number of biopsied blastomeres. However, this method requires technical skill, is time consuming, and is subject to false positives due to DNA contamination during the handling of PCR products.[41] Nonelectrophoretic PCR-sexing may be more practical and affordable for commercial embryo transfer programs. Embryos must be biopsied and exposed to the Ampli-Y solutions and run on a thermocycler. Sex can then be determined by ultraviolet transillumination, with male embryos indicating a pink fluorescence. This

technique has been shown to be highly accurate as well as significantly reduce or eliminate DNA contamination problems often seen with electrophoretic methods.[38]

Loop-mediated isothermal amplification (LAMP) is a DNA amplification method that relies on detection of a product by formation of a precipitate without the need for electrophoresis. When the target DNA fragments are amplified by LAMP, the turbidity of the reaction mixture may be measured by absorbance at 650 nm. Sexing is performed with a male-specific reaction using a tandem repeat sequence on the Y chromosome, and a male-female common reaction using the 1.715 satellite DNA sequence from biopsied blastomeres. Male embryos will test positive (absorbance ≥ 0.1) for both reactions, whereas female embryos will only test positive for the common reaction.[41]

`A study by Hasler, et al. found that transfer of biopsied embryos resulted in reduced pregnancy rates compared to similar quality intact embryos; therefore, the number of cells extracted from the embryo should be minimized.[38] LAMP has been performed using a single blastomere from a morula, although the removal of more than one cell will increase the accuracy of the procedure. A study by Hirayama, et al. found that LAMP-based sexing required less than one hour to complete and was a reliable method for use in the field. The accuracy of correct sex determination ranged from 75 to 100%, depending on the number of cells used.[41]

Recently, Sturmey, et al. reported that there were significant differences in metabolite profiles of preimplantation embryos. In that study, in vitro produced bovine blastocysts had sex-specific differences in the metabolic profile for 7 out of 18 amino acids that were profiled.[42] These results suggest that amino acid profiling may offer a noninvasive, prospective method of determining embryo sex in conjunction with other biochemical markers of embryo viability. This method, however, will require more research before its full potential can be realized in cattle.

Preimplantation genetic diagnosis

Marker assisted selection (MAS) can be used to screen embryos for certain marker genes which may be associated with favorable or unfavorable traits. This method is particularly effective with single gene targets such as those genes associated with coat color, bovine leukocyte adhesion deficiency (BLAD), and deficiency of uridine monophosphate synthase (DUMPS).[43]

Since a single cell biopsy would only contain one copy of the genome, and selection of preimplantation embryos through genetic screening will require the ability to identify numerous loci, whole genome amplification can be performed to augment the material available for genetic analyses.[44] Currently, there are two major methods of whole genome amplification: primer extension pre-amplification and nested PCR to simultaneously amplify regions of interest. Whole genome amplification is not completely reliable, but it is essential for further advancements in microchip analysis. Allelic discrimination is a technique that involves fluorogenic, sequence-specific probes to discriminate between certain alleles. The reliability of the genotyping based on nested PCR and allelic discrimination for two markers associated with milk production and one marker for gender has been reported.[45] In their study, the accuracy ranged from 97.8 to 99.3% across the three markers for comparing the biopsy genotype to the genotype of the remainder of the embryo.[45] An earlier study examined the genotyping efficiency of sex determination and three genetic markers using a combination of techniques, and reported genotyping efficiencies of 88 to 91%.[46]

The development of quantitative real-time PCR has allowed assessment of not only qualitative aspects of gene expression but also quantitative changes in gene expression in response to altered physiological states.[47] More recently, microarray technology has emerged as a method to scan an entire genome for variations in mRNA levels associated with a phenotype.[48] El-Sayed, et al. used microarray analysis to identify 52 genes that were differentially expressed between in vitro produced embryos that resulted in calf delivery following embryo transfer compared to those that failed to produce a pregnancy. Biopsies from embryos that resulted in calf delivery after embryo transfer had increased expression of mRNAs involved in implantation (*COX2, CDX2*), carbohydrate metabolism (*ALOX15*), cell signaling (*BMP15*) and signal transduction (*PLAU*). In contrast, embryos that did not result in pregnancy had high levels of mRNA transcripts involved in inflammation (*TNF*), implantation inhibition (*CD9*) and glucose metabolism (*AKR1B1*). In addition, they had higher levels of expression for transcription factors involved in programmed cell death or tumor formation (*MXS1, PTTG1*).[49] Several other studies, summarized in Table 3, have reported candidate genes whose transcripts or products may be involved in embryo survival or that are differentially regulated between in vivo and in vitro produced embryos.[50-60]

Metabolic assays

Metabolic assays are often non-invasive and may offer practitioners more quantifiable and objective markers of embryo characteristics. However, it should be noted that the in vitro culture conditions used during metabolic assays may skew the results. Preimplantation embryo development can be divided into two phases based on oocyte metabolic activity and embryonic control. The first phase is characterized by low metabolic activity where early cleavage development is controlled by the mRNAs and proteins from the oocyte. The second phase demonstrates a dramatic increase in metabolic activity, as the embryonic genome gains control of developmental processes following the maternal-zygotic transition.[2] Embryo viability should be assessed after the embryonic genome is activated, to ensure any observed differences reflect the physiology of the embryo and not that of the oocyte alone.[2]

Metabolic evaluation of developmental competence can include the measurement of nutrient uptake and metabolism, oxygen uptake, or embryo secretions. There are several non-invasive techniques that can be utilized to measure embryo metabolism. To measure the uptake and release of glucose, lactate, and pyruvate, embryos can be incubated in 1- to 4-μL droplets of culture medium for a few hours, and substrate concentrations can be calculated and compared with controls by measuring fluorescence of the reduced nucleotides NADH and NADPH. A disadvantage of this technique is the delay between the incubation period and the evaluation of nutrient uptake and release.

The metabolism of substrates such as glucose, glutamine, and pyruvate can also be evaluated and linked to developmental competence of embryos.[2] Briefly, embryos are incubated in the presence of one or two labeled substrates in a drop of culture medium containing bicarbonate. Radioactive CO_2 and H_2O produced during incubation, by metabolism of raidolabeled substrates, will be trapped in the bicarbonate solution. The resulting radioactivity can be measured to assess relative metabolic pathway activity of the embryo. Unfortunately, this method of assessment is a terminal procedure because it involves the use of radioactivity and possible incorporation of radioactive substrates into the embryo which could alter further development. In addition, this technique does not provide a means to assess the use of endogenous stores of the substrates.[2] Based on studies utilizing metabolic substrate analysis, in vitro produced blastocysts have a 2-fold higher production of lactate and much higher rates of oxidation of glucose, pyruvate, and lactate compared to in vivo derived blastocysts.[61]

Analysis of amino acid turnover within an embryo offers another technique for evaluating viability. Studies with human embryos have found that those which developed to the blastocyst stage had a lower range of amino acid turnover values than embryos that failed to develop.[62] The "Quiet Embryo Hypothesis" described by Leese in 2002 proposes that viable preimplantation embryos have a metabolism that operates within a lower range compared to that for non-viable or low-viability embryos.[63] Sturmey, et al. found that although bovine embryo amino acid profiles for in vivo- and in vitro-derived embryos were qualitatively similar, embryos produced in vitro had more variation and generally higher rates of amino acid turnover. Furthermore, they found that a greater proportion of embryos in the low metabolic activity range reached the blastocyst stage compared to those in the high metabolic activity range. These data support the quiet embryo hypothesis. Furthermore, it is likely that in vitro produced embryos are subject to more stress due to suboptimal culture conditions. This could result in greater metabolic activity as the embryo consumes greater quantities of nutrients/amino acids to carry out repair pathways.[42] The observation that the extent of DNA damage correlates with amino acid turnover in cattle preimplantation embryos is also consistent with the Quiet Embryo Hypothesis.[64] Amino acid profiling, thus, may be a useful noninvasive biomarker of early embryo developmental competence.

Currently, there is a trend away from single metabolite target analysis and towards more comprehensive metabolite profiling techniques. In metabolomics, selected groups of compounds and their metabolic intermediates are examined through analysis of the metabolome. Here the metabolome is defined as "the dynamic quantitative complement of all low molecular weight molecules (<1000 Da) present in cells at a particular physiological or developmental stage".[65] Low molecular weight metabolites are the end products from cellular regulatory activities and can serve as a measure of the embryo's physiological response to changes in gene expression or environmental conditions. Metabolomic analysis is currently limited because experimental methods must contend with quantifying and characterizing a wide diversity of metabolites without a method for amplification.[65]

Oxygen consumption is one of the best indicators of overall metabolic activity, as it is a measurement of the capacity of an embryo to produce ATP by oxidative phosphorylation. Embryo respiration can be evaluated by measuring either a decrease in oxygen or an increase in carbon dioxide into the microenvironment.[1] The developing embryo relies heavily on this process during the cleavage and blastocyst stages. Respiration rate and morphological quality were correlated, as higher quality embryos demonstrate the highest oxygen consumption, whether the embryos had been produced in vivo or in vitro. Furthermore, there was a positive correlation between respiration rate and pregnancy success.[66]

Lopes, et al. have developed and validated a high-resolution microsensor system to measure embryo respiration, which offers an alternative, non-invasive method of individual embryo quantification. The nanorespirometer measures the linear steady-state oxygen gradient produced by an embryo. Average respiration rates for in vitro produced bovine Day 7 embryos were 1.3 ± 0.1 nanoliters per hour.[67] Respiration rates were directly influenced by embryo diameter, which in itself is affected by embryonic stage and morphology. For Day 7 blastocysts, respiration rates increased proportionally in embryos of higher morphological quality and larger diameter, with the highest quality embryos having significantly higher respiration rates.[67] In vivo produced embryos with high oxygen consumption levels tended to have numerically higher pregnancy rates compared to pregnancy rates following transfer of embryos with lower oxygen consumption rates. However, this difference in pregnancy rates was not significant; likely due to the small sample size.[68] Although further studies are needed to confirm the reliability of this method, perhaps a combination of oxygen consumption and morphological evaluation may improve embryo selection methods.

Resources on embryo evaluation

Readers who are interested in additional information on evaluation of embryos with photographic illustrations are referred the *Manual of the International Embryo Transfer Society: A procedural guide and general information for the use of embryo transfer technology emphasizing sanitary precautions* (CD-ROM).[7] There is also a tutorial on embryo evaluation, *Bovine in vivo embryo slide set tutorial*, which should be available at http://www.iets.org/ in the near future.

Summary

Visual assessment of morphology remains the industry standard for evaluation of bovine embryos produced in vivo or in vitro. Embryo quality grade has been repeatedly shown to be highly predictive of pregnancy rates after embryo transfer. Embryos produced in vitro have unique morphological and developmental characteristics. The transfer of in vitro produced embryos may result in normal fetuses and calves, or may result in more severe problems including increased rates of abortion, dystocia, and neonatal death. Prior to embryo transfer, biopsy can provide early insight into embryo sex and its genetic profile and serves as the basis for application of more advanced molecular technologies that can be used to better identify developmentally competent embryos. Metabolic assays can offer an additional method of embryo assessment, but they will require additional development before they will be practical for commercial application. As genetic testing and metabolic assays become more efficient, the use of molecular markers will likely become increasingly valuable for assessment of embryo viability in cattle.

References

1. Overstrom EW: In vitro assessment of embryo viability. Theriogenology 1996;45:3-16.
2. Donnay I, Partridge RJ, Leese HJ: Can embryo metabolism be used for selecting bovine embryos before transfer? Reprod Nutr Dev 1999;39:523-533.
3. Lindner GM, Wright Jr RW: Bovine embryo morphology and evaluation. Theriogenology 1983;20:407-416.
4. Hasler JF, McCauley AD, Lathrop WF, et al: Effect of donor-embryo-recipient interactions on pregnancy rate in a large-scale bovine embryo transfer program. Theriogenology 1987;27:139-168.
5. Shea BF: Evaluating the bovine embryo. Theriogenology 1981;15:31-42.
6. Stringfellow DA, Seidel SM, editors: Manual of the international embryo transfer society. 3rd ed. Savoy (IL): International Embryo Transfer Society; 1998.
7. Stringfellow DA, Givens MD, editors: Manual of the international embryo transfer society. 4th ed. Champaign (IL): International Embryo Transfer Society; 2010.
8. Hasler JF, Henderson WB, Hurtgen PJ, et al: Production, freezing and transfer of bovine IVF embryos and subsequent calving results. Theriogenology 1995;43:141-152.
9. Stroud B, Hasler JF: Dissecting why superovulation and embryo transfer usually work on some farms but not on others. Theriogenology 2006;65:65-76.
10. Van Soom A, Vanroose G, de Kruif A: Blastocyst evaluation by means of differential staining: a practical approach. Reprod Domest Anim 2001;36:29-35.
11. Wright JM: Non-surgical embryo transfer in cattle embryo-recipient interactions. Theriogenology 1981;15:43-56.
12. Reichenbach HD, Liebrich J, Berg U, et al: Pregnancy rates and births after unilateral or bilateral transfer of bovine embryos produced in vitro. J Reprod Fertil 1992;95:363-370.
13. Looney CR, Lindsey BR, Gonseth CL, et al: Commercial aspects of oocyte retrieval and in vitro fertilization (IVF) for embryo production in problem cows. Theriogenology 1994;41:67-72.
14. Farin PW, Farin CE: Transfer of bovine embryos produced in vivo or in vitro: survival and fetal development. Biol Reprod 1995;52:676-682.

15. Hasler JF: Factors affecting frozen and fresh embryo transfer pregnancy rates in cattle. Theriogenology 2001;56:1401-1415.
16. Block J, Bonilla L, Hansen PJ: Effect of addition of hyaluronan to embryo culture medium on survival of bovine embryos in vitro following vitrification and establishment of pregnancy after transfer to recipients. Theriogenology 2009;71:1063-1071.
17. Farin PW, Britt JH, Shaw DW, et al: Agreement among evaluators of bovine embryos produced in vivo or in vitro. Theriogenology 1995;44:339-349.
18. Farin PW, Slenning BD, Britt JH: Estimates of pregnancy outcomes based on selection of bovine embryos produced in vivo or in vitro. Theriogenology 1999;52:659-670.
19. Grisart B, Massip A, Dessy F. Cimematographic analysis of bovine embryo development in serum free oviduct conditioned medium. J Reprod Fertil 1994;101:257-264.
20. Shamsuddin M, Larsson B, Gustafsson H, et al: Comparative morphological evaluation of in vivo and in vitro produced bovine embryos. Proc 12th Int Cong Anim Reprod 1992;3:1333-1335.
21. Van Soom A, Mateusen B, Leroy J, et al: Assessment of mammalian embryo quality: what can we learn from embryo morphology? Reprod Biomed Online 2003;7:664-670.
22. Iwasaki S, Yoshiba N, Ushijima H, et al: Morphology and proportion of inner cell mass of bovine blastocysts fertilized in vitro or in vivo. J Reprod Fertil 1990;90:279-284.
23. Farin CE, Hasler JF, Martus NS, et al: A comparison of Menezo's B2 and tissue culture Medium-199 for in vitro production of bovine blastocysts. Theriogenology 1997;48:699-709.
24. Crosier AE, Farin PW, Dykstra MJ, et al: Ultrastructural morphometry of bovine blastocysts produced in vivo or in vitro. Biol Reprod 2001;64:1375-1385.
25. Crosier AE, Farin PW, Dykstra MJ, et al: Ultrastructural morphometry of bovine compact morulae produced in vivo or in vitro. Biol Reprod 2000;62:1459-1465.
26. Keefer CL, Stice SL, Matthews DL: Bovine inner cell mass cells as donor nuclei in the production of nuclear transfer embryos and calves. Biol Reprod 1994;50:935-939.
27. Behboodi E, Anderson GB, BonDurant RH, et al: Birth of large calves that developed from in vitro-derived bovine embryos. Theriogenology 1995;44:227-232.
28. Edwards JL, Schrick FN, McCracken MD, et al: Cloning adult farm animals: a review of the possibilities and problems associated with somatic cell nuclear transfer. Am J Reprod Immunol 2003;50:113-123.
29. Miles JR, Farin CE, Rodriguez KF, et al: Angiogenesis and morphometry of bovine placentas in late gestation from embryos produced in vivo or in vitro. Biol Reprod 2004;71:1919-1926.
30. Farin PW, Piedrahita JA, Farin CE: Errors in development of fetuses and placentas from in vitro-produced bovine embryos. Theriogenology 2006;65:178-191.
31. Piedrahita JA, Wells DN, Miller AL, et al: Effects of follicular size of cytoplast donor on the efficiency of cloning in cattle. Mol Reprod Dev 2002;61:317-326.
32. Hill JR, Burghardt RC, Jones K, et al: Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. Biol Reprod 2000;63:1787-1794.
33. Sinclair KD, Kuran M, Sorohan P, et al: Dietary nitrogen metabolism, folliculogenesis and IVP in cattle. Theriogenology 1999;51:309.
34. Powell K, Rooke JA, McEvoy TG, et al: Zygote donor nitrogen metabolism and in vitro embryo culture perturbs in utero development and IGF2R expression in ovine fetal tissues. Theriogenology 2006;66:1901-1912.
35. Farin CE, Farmer WT, Farin PW: Pregnancy recognition and abnormal offspring syndrome in cattle. Reprod Fertil Dev 2010;22:75-87.
36. Shea BF: Determining the sex of bovine embryos using polymerase chain reaction results: a six-year retrospective study. Theriogenology 1999;51:841-854.
37. Bredbacka P: Progress on methods of gene detection in preimplantation embryos. Theriogenology 2001;55:23-34.
38. Hasler JF, Cardey E, Stokes JE, et al: Nonelectrophoretic PCR-sexing of bovine embryos in a commercial environment. Theriogenology 2002;58:1457-1469.
39. Schenk JL, Suh TK, Seidel GE Jr: Embryo production from superovulated cattle following insemination of sexed sperm. Theriogenology 2006;65:299-307.
40. Xu J, Guo Z, Su L, et al: Developmental potential of vitrified Holstein cattle embryos fertilized in vitro with sex-sorted sperm. J Dairy Sci 2006;89:2510-2518.
41. Hirayama H, Kageyama S, Moriyasu S, et al: Rapid sexing of bovine preimplantation embryos using loop-mediated isothermal amplification. Theriogenology 2004;62:887-896.
42. Sturmey RG, Bermejo-Alvarez P, Gutierrez-Adan A, et al: Amino acid metabolism of bovine blastocysts: a biomarker of sex and viability. Mol Reprod Dev 2010;77:285-296.
43. Hansen PJ, Block J: Towards an embryocentric world: the current and potential uses of embryo technologies in dairy production. Reprod Fertil Dev 2004;16:1-14.
44. Virta J, Markola J, Peippo J, et al: Sex determination of bovine embryo blastomeres by fluorogenic probes. Theriogenology 2002;57:2229-2236.
45. Peippo J, Viitala S, Virta J, et al: Birth of correctly genotyped calves after multiplex marker detection from bovine embryo microblade biopsies. Mol Reprod Devel 2007;74:1373-1378.

46. Chrenek P, Boulanger L, Heyman Y, et al: Sexing and multiple genotype analysis from a single cell of bovine embryo. Theriogenology 2001;55:1071-1081.
47. Sirard MA, Dufort I, Vallee M, et al: Potential and limitations of bovine-specific arrays for the analysis of mRNA levels in early development: preliminary analysis using a bovine embryonic array. Reprod Fertil Dev 2005;17:47-57.
48. Niemann H, Carnwath JW, Kues W: Application of DNA array technology to mammalian embryos. Proc Int Conf Farm Anim Reprod 2007;68:S165-S177.
49. El-Sayed A, Hoelker M, Rings F, et al: Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. Physiol Genomics 2006;28:84-96.
50. Wrenzycki C, Herrmann D, Carnwath JW, et al: Expression of the gap junction gene connexin43 (Cx43) in preimplantation bovine embryos derived in vitro or in vivo. J Reprod Fertil 1996;108:17-24.
51. Eckert J, Niemann H: mRNA expression of leukaemia inhibitory factor (LIF) and its receptor subunits glycoprotein 130 and LIF-receptor-beta in bovine embryos derived in vitro or in vivo. Mol Hum Reprod 1998;4:957-965.
52. Wrenzycki C, Herrmann D, Keskintepe L, et al: Effects of culture system and protein supplementation on mRNA expression in pre-implantation bovine embryos. Hum Reprod 2001;16:893-901.
53. Wrenzycki C, Wells D, Herrmann D, et al: Nuclear transfer protocol affects messenger RNA expression patterns in cloned bovine blastocysts. Biol Reprod 2001;65:309-317.
54. Rizos D, Lonergan P, Boland MP, et al: Analysis of differential messenger RNA expression between bovine blastocysts produced in different culture systems: implications for blastocyst quality. Biol Reprod 2002;66:589-595.
55. Lazzari G, Wrenzycki C, Herrmann D, et al: Cellular and molecular deviations in bovine in vitro-produced embryos are related to the large offspring syndrome. Biol Reprod 2002;67:767-775.
56. Bertolini M, Beam SW, Shim H, et al: Growth, development, and gene expression by in vivo- and in vitro-produced day 7 and 16 bovine embryos. Mol Reprod Dev 2002;63:318-328.
57. Wrenzycki C, Lucas-Hahn A, Herrmann D, et al: In vitro production and nuclear transfer affect dosage compensation of the X-linked gene transcripts G6PD, PGK, and Xist in preimplantation bovine embryos. Biol Reprod 2002;66:127-134.
58. Wrenzycki C, Niemann H: Epigenetic reprogramming in early embryonic development: effects of in vitro production and somatic nuclear transfer. Reprod Biomed Online 2003;7:649-656.
59. Wrenzycki C, Herrmann D, Lucas-Hahn A, et al: Gene expression patterns in in vitro-produced and somatic nuclear transfer-derived preimplantation bovine embryos: relationship to the large offspring syndrome? Anim Reprod Sci 2004;82-83:593-603.
60. Donnison M, Pfeffer PL: Isolation of genes associated with developmentally competent bovine oocytes and quantitation of their levels during development. Biol Reprod 2004;71:1813-1821.
61. Khurana NK, Niemann H: Energy metabolism in preimplantation bovine embryos derived in vitro or in vivo. Biol Reprod 2000;62:847-856.
62. Houghton FD, Hawkhead JA, Humpherson PG, et al: Non-invasive amino acid turnover predicts human embryo developmental capacity. Hum Reprod 2002;17:999-1005.
63. Baumann CG, Morris DG, Sreenan JM, et al: The quiet embryo hypothesis: molecular characteristics favoring viability. Mol Reprod Dev 2007;74:1345-1353.
64. Sturmey RG, Hawkhead JA, Barker EA, et al: DNA damage and metabolic activity in the preimplantation embryo. Hum Reprod 2009;24:81-91.
65. Singh R, Sinclair KD: Metabolomics: Approaches to assessing oocyte and embryo quality. Theriogenology 2007;68:S56-S62.
66. Lopes AS, Greve T, Callesen H: Quantification of embryo quality by respirometry. Theriogenology 2007;67:21-31.
67. Lopes AS, Larsen LH, Ramsing N, et al: Respiration rates of individual bovine in vitro-produced embryos measured with a novel, non-invasive and highly sensitive microsensor system. Reprod 2005;130:669-679.
68. Lopes AS, Madsen SE, Ramsing NB, et al: Investigation of respiration of individual bovine embryos produced in vivo and in vitro and correlation with viability following transfer. Hum Reprod 2007;22:558-566.

Table 1. Criteria for assigning quality grades to bovine embryos as recommended by the International Embryo Transfer Society.[7]

Code	Grade	Description
1	Excellent or Good	Symmetrical and spherical embryo mass with individual blastomeres (cells) that are uniform in size, color, and density. This embryo is consistent with its expected stage of development. Irregularities should be relatively minor, and at least 85% of the cellular material should be intact, viable embryonic mass. This judgment should be based on the percentage of embryonic cells represented by the extruded material in the perivitelline space. The zona pellucida should be smooth and have no concave or flat surfaces that might cause the embryo to adhere to a Petri dish or a straw.
2	Fair	Moderate irregularities in shape of the embryonic mass or in size, color and density of individual cells. At least 50% of the cellular material should be an intact, viable embryonic mass.
3	Poor	Major irregularities in shape of the embryonic mass or in size, color and density of individual cells. At least 25% of the cellular material should be an intact, viable embryonic mass.
4	Dead or Degenerating	Degenerating embryos, oocytes or 1-cell embryos: nonviable.

Table 2. Pregnancy rates of recipients following transfer of fresh bovine embryos of different stages of development and quality grades produced either in vivo or in vitro.

Embryo source	Stage of development	Percent pregnant (no. of transfers) by embryo grade			Reference
		Grade 1	Grade 2	Grade 3	
In vivo	Late morula + early blastocyst	71% (7)	56% (672)	44% (130)	Shea, 1981
	Morula to expanded blastocyst	64% (1748)	45% (438)	33% (100)	Wright, 1981
	Morula to expanded blastocyst	76%[a]	65%[a]	54%[a]	Hasler et al., 1995
	Morula to expanded blastocyst	73.2% (4163)	68.3% (3156)	56.3% (1641)	Hasler, 2001
In vitro	Late morula + blastocyst	54% (61)	51% (41)	26% (27)	Reichenbach et al., 1992
	Blastocyst	46.9%[b]	35.8%[b]	19.2%[b]	Looney et al., 1994
	Morula	56% (90)	43% (23)		Hasler et al., 1995
	Early to mid-blastocyst	56-58% (969)	41-46% (283)		
	Expanded to hatched blastocyst	50-62% (483)	56% (36)		
	Morula + blastocyst	18.5% (81)[c]			Block et al., 2009
		28.6% (70)[d]			
	Expanded blastocyst	38.3% (81)[c]			
		29.5% (78)[d]			

[a]320 total transfers among all 3 embryo grades; [b]813 total transfers among all 3 embryo grades; [c]Control group, and [d]Hyaluronan treated group.

Table 3. Potential genes for assessing development and function of bovine embryos.

Gene Function Gene Name	Gene Abbreviation	References
Growth factor & cell signaling		
Bos taurus bone morphogenetic protein 15	*BMP15*	El-Sayed et al., 2006
Bos taurus urokinase-type plasminogen activator	*PLAU*	El-Sayed et al., 2006
Leukemia inhibitory factor, beta receptor	*LIF, LR-β*	Eckert and Niemann, 1998
Insulin-like growth factor 1 receptor	*IGF1R*	Bertolini et al., 2002
Insulin-like growth factor 2 & receptor	*IGF2, IGF2R*	Bertolini et al., 2002
Fibroblast growth factor 2 (basic)	*FGF2*	Lazzari et al., 2002
Transcription factors		
Bos taurus msh homeobox 1	*MSX1*	El-Sayed et al., 2006
Homo sapiens pitutary tumor-transforming 1	*PTTG1*	El-Sayed et al., 2006
Caudal type homeobox transcription factor 2	*CDX2*	El-Sayed et al., 2006
Metabolism		
Bos taurus arachidonate 15-lipoxygenase	*ALOX15*	El-Sayed et al., 2006
Bovine aldose reductase family 1, member B1	*AKR1B1*	El-Sayed et al., 2006
Glucose transporter 1, 3, 4	*GLUT1,GLUT3, GLUT4*	Wrenzycki et al., 2001; Bertolini et al., 2002; Lazzari et al., 2002
Glucose-6-phosphate dehydrogenase	*G6PD*	Wrenzycki et al., 2002
Phosphoglycerate kinase 1	*PGK1*	Wrenzycki et al., 2002; El-Sayed et al., 2006
Bos taurus elongation factor 1 alpha 1	*EEF1A1*	El-Sayed et al., 2006
Maternal recognition & implantation		
Interferon tau	*IFNT*	Wrenzycki et al., 2001
Bos taurus prostaglandin G/H synthase-2	*COX2*	El-Sayed et al., 2006
Bos taurus CD9 antigen	*CD9*	El-Sayed et al., 2006
Embryo compaction/cavitation		
Connexin 31, 43	*CX31, CX43*	Rizos et al., 2002; Wrenzycki et al., 1996
Cadherin 1, type 1 (E-cadherin)	*CDH1*	Wrenzycki et al., 2001
Desmocollin 1, 2	*DSC1, DSC2*	Wrenzycki et al., 2001
Stress adaptation		
Bos taurus thioredoxin mRNA	*TXN*	El-Sayed et al., 2006
Copper/Zinc superoxide dismutase	*Cu/Zn-SOD*	Rizos et al., 2002
Heat shock protein	*HSP*	Wrenzycki et al., 2001
Other		
DNA methyltransferase 1, 3 alpha	*DNMT1, DNMT3A*	Wrenzycki et al., 2001; Wrenzycki and Niemann, 2003
Bos taurus tumor necrosis fator	*TNF*	El-Sayed et al., 2006
Mammalian achaete-scute homologue 2	*MASH2*	Wrenzycki et al., 2001
X-inactive specific transcript	*XIST*	Wrenzycki et al., 2002

Review of pregnancy diagnosis techniques in cattle and small ruminants

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Abstract

Pregnancy diagnosis is a common management practice for ruminants and there are a number of methods available, with each having advantage(s) and/or disadvantage(s). A brief review of pregnancy diagnosis techniques in cattle, goats, and sheep is provided.

Keywords: Cattle, goat, sheep, pregnancy, diagnosis

Introduction

Pregnancy diagnosis is a common management practice for the purpose of identifying pregnant and non-pregnant ruminants. Early and accurate pregnancy diagnosis is of considerable value in improving efficiency of production.[1] By discriminating between pregnant and non-pregnant animals, better management control is made possible. There are a number of methods available for diagnosis of pregnancy in ruminants and the method(s) of choice will depend on equipment availability, number of days post-breeding the animals are examined, and the desired accuracy. This manuscript describes different methods of pregnancy diagnosis in cattle and small ruminants and assesses and compares their usefulness.

Observation

Traditional methods of pregnancy diagnosis are presumptive based on observation of the animals and include non-return to estrus, abdominal enlargement, and onset of lactation. These methods are non-invasive and require only basic knowledge and training.

Non-return to estrus

In non-return to estrus, it is assumed that animals that are not observed to return to estrus within the normal interval (21 days average in cows and does, 17 days average in ewes) are pregnant. This approach is advantageous over the other two observational methods in that it occurs earlier in gestation and thereby limits the time before re-breeding, treatment, or culling of open animals. Provided that adequate time, records, and knowledge are applied, non-return to estrus yields the potential to be early and accurate. In the bovine, standing to be mounted is the most reliable indicator of heat and is referred to as 'standing heat'. Secondary signs include vulvar discharge, mounting of other cows, vocalization, restlessness, swelling or reddening of the vulva, and lip curling. In does, increased vocalization, tail-flagging, frequent urination, decreased appetite, decreased milk production, seeking of interaction with bucks, and standing to be mounted are all indicators of estrus. In ewes, a marking harness or teaser ram is most commonly used to aid in estrus detection.

Several techniques have been developed to aid in detection of heat to alleviate this problem. In a review of electronic devices used in cattle to identify estrus animals, Rorie, et al. reported that mount detectors, pedometry, and electrical resistance of reproductive tract fluids increase efficiency of heat detection compared to visual identification.[2] Estrus detection animals may serve as additional aids in estrus detection. While androgen treatment of steers, freemartins, and cows could be employed, its application is illegal in the United States. Instead, surgically-altered bulls are frequently used. Epididectomy, penile or preputial deviation, and penile or preputial fixation are the most common modifications in the preparation of teaser bulls. Tailhead markers also serve to indicate animals in standing heat. In goats, testosterone-treatment of wethers or does, epididectomized and vasectomized bucks, and intersex animals as well as marking harnesses and 'buck rags' act to aid in heat detection. Similarly, vasectomized rams with marking harnesses are frequently used in sheep. In one study of 177 ewes, a harnessed vasectomized ram raddled only 1 of 118 pregnant ewes (99% accuracy); however, only 31 of 59 (53%) non-pregnant animals were marked, demonstrating an the limited sensitivity of this test in detecting non-pregnant animals.[3]

Efficiency of heat detection (sensitivity) and accuracy (positive predictive value) serve as limitations in diagnosis pregnancy in this manner. In a study of estrus detection in dairy operations, the efficiency of detection is usually less than 50%; in beef cattle, detection rates are often even lower due to less frequent observation and less intensive management.[4] Similarly mediocre efficiency is also likely in sheep and goats. The length of estrus in cows is reported to range from 0.5-24 hours or 33 minutes to 35.8 hours, and 24% of dairy cows have estrus periods characterized as low intensity and short duration (<1.5 mounts per hour and <7 hours duration).[2,5] Duration in does

is 12-48 hours. Based on this information, it is possible to understand how animals in estrus could be overlooked. Other factors affect display of estrous behavior in cows, including type of housing, footing, lameness issues, number and reproductive status of herdmates, time of day, environmental temperature, nutrition, and level of milk production.[6] Indeed, malnutrition may be a common cause of failure to cycle, particularly in beef breeds at the end of the cold season. Similar limitations exist in small ruminants. Additionally, some does will not stand for particular bucks (especially if they are young or de-scented), even if the does are in heat. As all these factors contribute to the degree of estrous behavior displayed, they have the potential to limit efficiency of heat detection.

Accuracy may also be limited by confounding factors that prevent return to estrus. Cystic ovarian disease is the second most common reason (besides pregnancy) for failure to return to estrus in the bovine. Any other form of ovarian disease that alters cyclical activity may similarly result in lack of behavioral signs despite absence of pregnancy. Other reproductive abnormalities that disrupt the estrous cycle may also complicate diagnosis. Hydrometra, which has a reported incidence of 3.0 to 20.8% in dairy does, is one example of a condition that could lead to a false positive.[7] Additionally, sheep and goats show seasonal anestrous, and breeding prior to the transitional period or outside of the normal breeding season via a synchronization and induced-ovulation protocol limit the potential application of non-return to estrus in determination of reproductive status.

It should also be noted that false negatives may occur in using this criterion for determination of pregnancy status. Some females continue to exhibit signs of behavioral estrus despite being pregnant. This can be problematic as administration of prostaglandins or repeat insemination usually results in abortion. Additionally, teaser animals with very high libido may mount non-receptive animals; this is particularly significant when relying on marking to indicate estrus rather than observation of standing heat.

Weight gain

Weight gain, particularly abdominal weight in the third trimester, and abdominal contour may be used as indicators of pregnancy. In ewes at four months of gestation, a body weight increase of 6-12% was documented in animals carrying singletons and 13-16% in animals with twins.[8] While waiting for observation of this change is very non-invasive, serious limitations are evident. First of all, application of this approach implies a noteworthy delay in arriving at a presumptive diagnosis, and the cost of retaining and feeding an open animal while waiting for weight gain to manifest can be significant. Also, alternative causes of distended abdomen such as hydrometra or stretched abdominal muscles may account for apparent weight gain and lead to false positives. False negatives may also occur, particularly in primiparous females carrying a single fetus. In a review of 24 diagnostic methods used in sheep, increase in body weight was considered unreliable or unsatisfactory in its ability to identify pregnant animals.[9]

Onset of lactation

Onset of lactation or udder development may be used as suggestive signs of pregnancy. As with weight gain, a major drawback of reliance on this physiological change is that it does not occur until late gestation and therefore could result in significant economic losses through lost time and retention of open animals. It does exhibit some reliability. In a comparison of several methods of pregnancy diagnosis, at 130 days of gestation, 84% of ewes showed enlarged, firm udders and 14% demonstrated slight to moderate swelling.[3] Elimination of the dry period and continuous lactation may be applied in dairy cows or goats, as it does not appear to adversely affect milk yield in does and decreases metabolic imbalances despite a slight decrease in production in cows.[10] In these systems, onset of lactation or udder development is eliminated as a marker of pregnancy status. False positives may occur in the application of this approach. Inappropriate lactation syndrome has been described in small ruminants, with numerous anecdotal reports in addition to those in the literature.

Despite their relative ease of application, observational forms of pregnancy detection lack the sensitivity and specificity required for diagnostic precision. In fact, the errors and limitations probably make these tests more expensive in the long-term, through retention of open animals, as compared to other tests that cost more initially. Due to great advances over the last few decades, these approaches are being applied with decreasing frequency as techniques with lower error rates are adopted.

Palpation

Abdominal palpation/ballottement

Abdominal palpation for pregnancy diagnosis in ewes and does is a traditional management method used by producers throughout the world. Tentative diagnosis is done by balloting at the base of the udder.[11] The gravid uterus or fetus can sometimes be palpated through the abdominal wall by placing a hand on either side of the

abdomen and lifting upwards. If a fetus is felt to drop on the palpating hand the animal is pregnant.[11] This technique becomes easier and more reliable as pregnancy advances and is easier in thinner ewes and does. Pregnancy was diagnosed in does with a 76% accuracy after 80 days of gestation by the abdominal palpation method.[12] At 61 to 70 days of gestation, the accuracy of abdominal palpation method has been reported to be 70%.[11] Accuracies of 80 to 95% were reported in ewes at 90 to 130 days of pregnancy and it was estimated that an experienced operator could handle sheep at a rate of 200 per hour provided adequate assistance.[13] A fetus can sometimes be balloted low in the right flank during the last month of gestation. The examination can be made easier by withholding feed and water for at least 12 h before examining the animals. Since this method is simple and does not involve any equipment it can be used by sheep and goat owners to screen their flock. Although frequently used by sheep and goat owners this technique is not an acceptable method of pregnancy determination because of less than ideal accuracy and the inability to determine pregnancy status early.

Rectal-abdominal palpation

Rectal-abdominal palpation technique for diagnosing pregnancy has been used in the ewe and doe.[14,15] It is recommended that ewes and does be held off feed overnight prior to the examination to improve speed and accuracy of this technique. Animals are placed in dorsal recumbency (e.g., a laparotomy cradle) for examination. A soap enema is injected into the rectum and a lubricated rod with a rounded tip is inserted into the rectum to a depth of 30 to 35 cm.[14] The free hand is placed on the caudal abdomen while the rod is manipulated with the other hand. The rod is moved ventral and dorsal and from medial to lateral until an obstruction is encountered and palpated against the abdominal wall or a decision is reached that the ewe or doe is not pregnant. The method is approximately 97% accurate at 60 to 64 days post-mating and requires less than 30 seconds per ewe allowing potentially 200 ewes per hour to be examined using this technique.[14] This technique is 94 to 97% accurate in diagnosing pregnancy in does 55 days after breeding.[15] However, this procedure is not without risk. Rectal trauma, abortion, and even death have been reported following rectal-abdominal palpation.[14-18] It takes significantly more time to make a diagnosis in ewes which are not pregnant compared to those which are pregnant and the longer time taken for the non-pregnant animals may explain the higher incidence of perforation amongst them.[17]

Palpation of the uterus via laparotomy

The uterus can be palpated directly through a small laparotomy incision made cranial to the mammary gland and approximately 1.5 cm lateral to the midline.[19] The incision should be sufficient to allow the introduction of two fingers to palpate the uterus. An enlarged thin-walled uterus containing fluid is taken as positive evidence of pregnancy. Direct palpation of the uterus gave more than 92% accuracy in diagnosing pregnancy in ewes four to five weeks pregnant.[20] An experienced operator may detect cotyledons at 42 days after service. Direct palpation of the uterus approaches 100% accuracy after 42 days of gestation.[21] Although this method is very accurate, it is not applicable under field conditions due to surgical risks which reduce the subsequent fertility of animals being tested.[22] Its use is limited to experimental purposes or during abdominal exploratory for other purposes.

Palpation of the cervix

Palpation of the cervix involves digital palpation of the external cervical os per vagina at 50 days or more post-breeding. A very soft, blunted cervix or inability to reach the cervix is suggestive of pregnancy while a firm conical-shaped cervix projecting into the vagina is suggestive of non-pregnancy.[23] In addition to palpation of the cervix, cervical mucus examination has been described with limited success.[24] The Estroscope was used to determine changes in the color and flow elasticity of cervical mucus.[25] Changes in color of cervical mucus from clear and colorless to opaque and pale yellow along with increased elasticity were obvious by the fourth week of pregnancy.[24] Unfortunately, for both techniques similar observations would be made in pseudopregnant animals, and animals would be erroneously categorized as pregnant.

Transrectal palpation

Transrectal palpation of the uterine contents and the ovaries is the most common method used by veterinarians for diagnosis of pregnancy and ovarian structures in cattle. Transrectal palpation had been the standard method of detecting pregnancy in cattle until ultrasonography of the reproductive tract became more widespread.[26,27] However, transrectal palpation is still a very important diagnostic tool.

Palpation of the ovaries per rectum. Detection of a corpus luteum (CL) is important for making decisions regarding treatment of cattle. The CL persists as a result of the maternal recognition of pregnancy. The presence of a full-size (20 to 25 mm diameter), mature CL 20 to 22 days after a cow has been inseminated is suggestive of

pregnancy. Follow-up examinations conducted at a later date show this method to be 85 to 90% accurate as a means of predicting the outcome of pregnancy.[1] The limitations of this technique for the detection of pregnancy are the same as those which apply to the use of plasma or milk progesterone concentrations. Unfortunately the technique of transrectal palpation to detect a functional CL is inaccurate.[28] Previous studies have determined that the sensitivity of transrectal palpation for detection of CL's ranges from 33 to 96%, and specificity ranges from 22 to 100%.[28,29] Detection of ovarian structures by transrectal palpation requires skill and in general more experienced clinicians are more accurate than less experienced ones.[30] Lastly, there may be some risk of enucleating CL's during transrectal palpation of the ovaries thus causing the termination of a diagnosed pregnancy. Due to the inaccuracy of this technique and the risk of enucleating a corpus luteum of pregnancy, the authors do not recommend transrectal palpation for persistence of a CL to diagnose pregnancy in cattle.

Palpation of the uterus per rectum. The traditional method for diagnosis of pregnancy in cattle for the past 150 years has been examination of the uterus by transrectal palpation.[31] Descriptions of the technique have been published previously.[1,32] Palpation of the bovine uterus per rectum is one of the most frequent procedures performed and is the most frequent method used for pregnancy diagnosis and a skilled practitioner is able to detect pregnancy in cattle as early as day 35.[33,34] The importance of a systematic and non-traumatic technique of palpation per rectum cannot be overemphasized. Embryonic and fetal deaths can be induced accidentally by this procedure.[35-38] However, there is contradictory information regarding the potential deleterious effects of palpation per rectum for early pregnancy diagnosis on embryo/fetal viability with some studies suggesting a possible adverse[39-43] effect of early palpation per rectum and other studies suggesting that the time at which the first palpation per rectum was performed after insemination had little effect on the calving rate.[44,45] A positive diagnosis of pregnancy can be made by palpation and identification of: the amniotic vesicle (AV); the fetal membrane slip (chorioallantoic membrane); placentomes; and the fetus (Table 1).[1] The latter two positive signs are detected at an advanced stage when it is of less economic importance.

Palpation of the amniotic vesicle. The AV can be detected by transrectal palpation of the bovine uterus as early as 30 days of gestation when the AV is about 10 mm in diameter; by 35 days, it is about 17 mm in diameter. The uterine horn is palpated along its length from base to tip; the AV can be identified as a distinct spherical, turgid object floating in the allantoic fluid. From 30 to 60 days of gestation the AV can be detected by transrectal palpation of the bovine uterus and the diameter of the vesicle is useful in determining approximate stage of gestation (Table 1). Palpation of the AV may not be without risk as some studies have implicated it as a cause of atresia coli and jejuni in calves.[46,47]

Palpation of the chorioallantosis. From 35 to 40 days of gestation it is possible to palpate the chorioallantois (fetal membrane slip) in the horn ipsilateral to the AV and embryo of cattle. The uterine horn is grasped through the rectal wall between the thumb and a finger, and squeezed gently, allowing the grasped structures to fall away. The chorioallantoic membrane is the first to 'slip away', followed by the thicker uterine and rectal walls. The fetal membrane slip can be detected until about day 90 of gestation in the cow (Table 1). Unlike palpation of the AV, palpation per rectum of the chorioallantois for early pregnancy diagnosis is not associated with birth defects of calves.[48] Also, palpation per rectum for early pregnancy diagnosis in dairy cattle using fetal membrane slip does not affect embryo/fetal viability.[49]

Palpation of the fetus. From about 60 or 65 days of gestation, the AV loses its turgidity. It is therefore possible to palpate the fetus directly and estimate the stage of gestation from the size of the fetus (Table 1). However, the fetus is not always within the reach of the examiner. Depending on the size of the dam and the examiner, the fetus may be out of reach (descended into the abdomen) between 120 and 210 days of gestation. While the fetus is not accessible, other positive/cardinal signs of pregnancy must be sought to make a definitive diagnosis.

Palpation of the placentomes. Placentomes (caruncles and cotyledons) can first be palpated from about ten to 11 weeks of gestation as irregularities on the surface of the uterine wall, particularly along the greater curvature of the gravid horn. From three months onward, the placentomes can be identified as discrete structures, notably in the uterine body at the base of the horns just over the pelvic brim. Placentomes become progressively larger as the pregnancy advances until approximately day 150 (Table 1).

Indications/suggestions of pregnancy

From about 30 to 35 days of gestation, the uterine horn ipsilateral to the ovary with the CL increases in size relative to the non-gravid horn. At the same time, the uterine wall is thinner because of the accumulation of fluid within the uterus. Unfortunately other factors (e.g. post-partum uterus before complete involution, mucometra, hydrometra, and pyometra) can be responsible for a disparity in the size of the uterine horns.

The middle uterine artery provides the main blood supply to the gravid uterus. To satisfy the demands for increased blood during pregnancy, the middle uterine artery enlarges. As the artery becomes larger, the turbulence in the blood flow results in a change in the pulse character so that the artery feels as if it is 'buzzing' or vibrating; this is called fremitus. The uterine arteries are in the broad ligaments and can be located by palpating along the shaft of the ilium. Unilateral fremitus develops on the gravid side from about three to four months of gestation, and is likely to be bilateral from about six to seven months onward in a singleton pregnancy. There is variation in the time of onset and, in some animals, where the blood supply to the uterus is largely from one side, it may only be unilateral. Since fremitus does not develop until the third or fourth month of gestation (delays pregnancy determination) and other factors may be responsible for fremitus developing, fremitus alone should not be used to determine pregnancy status.

Imaging

The application of various imaging modalities in pregnancy diagnosis and monitoring is a mainstay in human reproductive medicine and has been increasingly adapted to animal application. The benefits are numerous and include direct visualization of the fetus and heart rate; monitoring of viability; determination of fetal number, gender, and gestational age; and identification of pathological processes and fetal death. Great advances have been made–especially regarding ultrasonography–in the preceding decades, leading to an increase in its utilization in veterinary medicine.

Radiography

Radiography can be applied for suggestive diagnosis based on the presence of a fluid-filled uterus and as a definitive diagnosis after ossification of fetal bones occurs which allows direct visualization of the fetus. Its use is limited to impossible in the bovine due to the sheer size of the abdomen and necessity of expensive equipment and near-dangerous levels of x-rays required to achieve an image; however, it can be used in small ruminants to diagnose pregnancy and determine fetal numbers. The work of Benzie achieved 96% accuracy from day 43 of gestation in black face sheep.[50] A study in Clun Forest ewes suggests accuracy may be lower–closer to 79%–in larger ewes; however, greater than 90% accuracy was obtained in determining number of lambs after day 90 of gestation.[51] In Saanen and Toggenburg goats, ossification and fetal skeleton may be observed as early as day 58 and are more reliably evident by day 65; it is suggested that radiographs after day 70 can lead to accuracy in diagnosis of pregnancy and fetal numbers approaching 100%.[52] Alternately, it has been recommended to wait until day 90 in the doe to decrease false negatives; hydrometra may also be diagnosed at this point if the uterus is large and fluid-filled without detection of fetal skeletons.[53]

Radiography is highly accurate in pregnancy diagnosis. The ability to determine fetal number should not be underestimated in its importance, especially in small ruminants, as it allows identification of ewes and does carrying multiples and subsequent implementation of nutritional management to minimize risk of metabolic disorders (pregnancy toxemia). Disadvantages include expense of equipment and procedure, radiation exposure, limited applicability in larger ewes and does, and necessity of waiting until late in gestation (following fetal ossification).

Ultrasonography

Great strides have been made in the application of ultrasonography to pregnancy diagnosis in ruminants over the last several decades. Decreasing cost and increasing availability of equipment; potential for earlier diagnosis of pregnancy; immediate diagnosis with performance of the procedure; and ability to determine fetal gender, viability, approximate fetal age, and existence of some maternal and fetal abnormalities have led to commonplace use of ultrasound in reproductive applications. In addition, transrectal ultrasound is very safe.[54,55] An experienced operator can work very efficiently, with reports that up to 300 animals per hour may be examined (60 to 120 if looking for number of fetuses) with transabdominal scanning; transrectal viewing requires more time.[56] While cost may still limit its use in production animal medicine, decreasing cost of technology coupled with increasing applicability is likely to ensure growing application in the future.

A-mode. A-scan ultrasonography relies on the differential reflection of sound waves, particularly fluid versus tissue, and it has been applied in the past to diagnose pregnancy. A characteristic light, sound, or oscilloscopic blip pattern is produced by the unit with the detection of a fluid-filled structure; when encountered in the caudal abdomen, a diagnosis of pregnancy is made. It displays some reliability in sheep and goats.[3] Additional advantages include use as early as 40 to 50 days of gestation and accuracy approaching 95% at 60 to 80 days of gestation.[57,58] The shortcomings of this method are evident in a potentially high rate of false positives, such as those that may be misdiagnosed due to a full urinary bladder, pyometra, or hydrometra.[59]

Doppler. Detection of pregnancy via the application of the Doppler principle to detect fetal pulse has been applied historically. This technique relies on changes in sound waves produced when they are reflected off of moving objects (blood, heart wall, etc.). Detection of the middle uterine artery, fetal heartbeat, umbilical cord, and fetal movement may be indicators of pregnancy with this procedure.[11] It is safe and reliable and has been applied successfully in cows, ewes, and does.[60] In comparison to A-mode ultrasonography, it is more accurate and allows earlier pregnancy diagnosis in ewes and it also allows detection of multiple fetuses, albeit with low accuracy.[60] Transrectal application in goats achieved 94 to 100% correct diagnoses.[15] However, it has fallen out of favor with increasing utilization of B-mode ultrasound due to the many advantages it presents.

B-mode ultrasound. The observation of real-time images based upon the differential reflection of sound waves to an ultrasound probe that serves to both emit and receive signals is the most clinically applicable (and currently the most regularly-utilized) form of ultrasonography applied in reproductive management. Most frequently, a 5.0 to 8.0 MHz linear array or curvilinear probe is employed; 3.5 MHz probes are most useful for transabdominal use, particularly in the bovine, due to their greater tissue penetration. Both transrectal and transabdominal (in the right flank) approaches are described. The transrectal approach has been employed in all ruminant species. In the bovine, the probe can be carried in manually after removing fecal material while the transducer is usually fixed to a rigid extension rod for use in small ruminants. Because the uterus remains in the pelvic cavity during early gestation, this technique is advantageous in that it allows earlier detection of pregnancy. The transabdominal approach is performed high in the right flank. Some operators restrain animals in dorsal or lateral recumbency for this procedure, but it can be performed standing, which is more clinically useful.

In the bovine, with a 5.0 MHz transducer applied transrectally, an AV can be detected at day 13 to 14 and an embryo by day 26 to 29.[61] Accuracy may approach 100% by day 22 in ideal circumstances, and heifers may be diagnosed up to three days earlier than cows.[62,63] It is suggested that a 5.0 MHz transrectal probe be used after day 24 in the cow, such that a heartbeat may be detected. More recently, monitoring for CL regression by day 20 has been investigated as a potential indicator of pregnancy; greater than 25% regression from day 14 to day 20 may be considered as diagnostic of not pregnant while less than 10% regression denotes pregnancy.[64] This approach has only recently gained attention and is not yet suitable for routine application; however, it is noteworthy in that it allows diagnosis prior to return to estrus and may allow earlier re-breeding. Transcutaneous ultrasound results in very low sensitivity in early pregnancy diagnosis compared to transrectal ultrasound, but it may be used as a non-invasive technique in mid- to late-gestation.[65] In addition to identifying pregnant animals quickly and non-invasively, this approach allows assessment of uterine fluids, placenta, vasculature, and the fetus which may identify potential pathologies.[66]

Generally, in small ruminants, fluid pockets in the uterus representing the embryonic vesicle are evident by transrectal ultrasonography at day 20 of pregnancy, the beginning of formation of placentomes at day 21, and the embryo proper at day 25 to 30. However, visualization of the fluid in membranes has been reported as early as day 15 and observation of heartbeat at days 18 to 19; with a 7.5 MHz transrectal probe, all sheep embryos were counted accurately at day 25.[67] In goats, transrectal ultrasound can reach 98.7% sensitivity and 100% specificity at day 26.[68] Similarly, transrectal scanning of sheep results in high accuracy from days 24 to 34, with specificity of 98% for days 32 to 34.[69] Another report indicated accuracy may reach 99% in the ewe at least 45 days post-breeding.[70] Buckrell suggested that day 45 to 50 may be the ideal time to diagnose pregnancy in sheep and goats.[71] Some studies suggest that after day 25, the transabdominal method is more accurate than transrectal in determining reproductive status.[72] Because transabdominal ultrasound may be done even as the uterus falls over the pelvic brim into the abdomen, it is suggested days 40 to 75 in goats and days 45 to 90 in sheep are acceptable periods to obtain a high degree of accuracy.[53,73] When transrectal ultrasound is used, fasting the animals for 12 hours and lifting the abdomen may significantly improve diagnostic accuracy in the very early (day 18 to 24) and later (day 41 to 50) periods.[74]

Identification of fetal number is important, especially as it aids in nutritional management to reduce impact of metabolic disorders associated with multiple fetuses. It is possible to recognize twins (and sometimes triplets or quadruplets) via ultrasound in all ruminants as the presence of multiple embryonic vesicles or fetuses. Fowler and

Wilkins determined multiple versus single pregnancies with 97% accuracy in sheep; however, recognition of twins versus triplets was less accurate than determination of singles versus multiples, and ewes were restrained in dorsal recumbency and clipped for their analysis.[75] In small ruminants, it is recommended that days 45 to 100 are optimal for estimation of fetal number.[58,75,76]

Fetal gender determination, or fetal sexing, is useful in that it allows planning for future offspring and marketing of animals that are still in utero. In cows, Muller and Wittkowski described fetal gender determination from 73 to 120 days of gestation with 94% accuracy based on observation of the mammary teats or scrotum.[77] Curran, et al. reported earlier diagnosis, at day 55 to 60, based on location of the genital tubercle.[78] The genital tubercle can be recognized as a hyperechoic, bi-lobed structure that migrates to a position between the hindlimbs beneath the tail in the female and just caudal to the umbilicus in the male. In small ruminants, the two lobes may not be discernable, and the genital tubercle may appear as a single echogenic spot on ultrasound; nonetheless, it migrates similarly.[79] Most application has focused on this structure for fetal gender determination. Ali found gender determination possible in all cases in 15 multiparous Friesian cows from days 56 to 98 of gestation, with an overall accuracy of 97.3%.[80] Accuracy of fetal gender determination in goats is highest at 40 to 60 days gestation, with 100% and 85.7% accuracy in singletons and multiple pregnancies, respectively.[81] However, genital tubercle migration may show breed and individual variation, so it is usually suggested that diagnosis be sought between 55 and 70 days of gestation. Also, genital tubercle migration can occur significantly later in animals produced by embryo transfer as compared to those resulting from natural mating.[82] Sex determination can also be carried out by the transabdominal approach, but with both methods, position of fetus can hinder diagnosis due to difficulty visualizing the necessary structures.[80,83]

Estimation of gestational stage and development is also possible and provides a potential benefit of ultrasound. Crown-rump length, bi-parietal diameter, eye-socket diameter, trunk diameter, head length, nose diameter, abdominal diameter, umbilical diameter, and meta-carpal length have been applied with some success in estimating fetal age.[84-88] Simultaneous measurement and application of two different measurements of bones with different growth allometry can increase estimate reliability.[89] Transcutaenous ultrasound may be sufficient to identify structures to aid in estimation late in gestation.[88] Crown-rump length and bi-parietal diameter as well as thoracic, skull, and placentome measurements are correlated with developmental stage in the goat.[81,86] The relationship of placentome area to gestational age is stronger in does as compared to ewes.[90] Full discussion of findings and regression equations used in stage determination is beyond the scope of this article, but it merits mention that the ability to estimate provides an advantage of pregnancy diagnosis via ultrasonography in cases where exact breeding date is unknown. Furthermore, tracking of fetal development and comparison to established normal values can be particularly useful in the area of advanced reproductive technologies. For further information on the topic, the reader is referred elsewhere.[81]

Observation of the fetus and evaluation for viability and potential abnormalities is a particularly useful aspect of ultrasound in comparison to the other modalities. Irregular contours of the developing embryo or fetus, debris on surfaces or in fluid, breaks in the amniotic membrane, and reduction in fluid volume are a few abnormalities associated with death of the developing conceptus.[91] Cessation of embryonic heartbeat and subsequent loss has been documented in studies of early diagnosis.[87] Abnormalities associated with late gestation and associated ultrasound findings have been reviewed.[92] Hydrallantois, enlarged placentomes, hyperechoic debris, fetal inactivity, and large offspring syndrome are abnormalities that may be recognized on ultrasound. For example, a study involving serial scans allowed identification of hydrops allantois in a ewe at 110 days gestation.[72] This is especially important in its application to advanced reproductive technologies (cloning, in-vitro fertilization, etc.), which are associated with increased incidence of some of the aforementioned issues. It also merits mention that ability to view the uterus and ovaries may allow identification of pathological processes in the non-pregnant animal, many of which may have direct bearing on fertility.[93]

A final note is made that the ability to use color flow Doppler is drawing attention as it may apply to pregnancy diagnosis. Significant changes in blood flow are evident in the pregnant versus the non-pregnant uterus at day 18 of gestation[94] and attempts have been to correlate CL blood flow to pregnancy status in embryo recipients (with low sensitivity and specificity thus far).[95] Currently, these approaches are interesting from a research standpoint but they do not yet have a clinical application. Nonetheless, it will be interesting to observe their development and information they may yield in the future.

There are multiple advantages of using ultrasound to determine pregnancy status, and very accurate results may be obtained by the experienced practitioner. The ability to directly view the embryo or fetus imparts important information regarding its stage, viability, and gender as well as potential recognition of the presence of multiples.

Chemical assays

Estrone sulfate

Estrone sulfate (ES) is the major estrogen produced by the fetoplacental unit during pregnancy and is conjugated in the fetal liver. Measurement of ES has been used to diagnose pregnancy in cattle, goats, and sheep.[96-98] Estrone sulfate levels in maternal plasma of cattle are undetectable until approximately day 72 of gestation.[99] Measurement of ES is not a reliable method of pregnancy diagnosis in cattle until after 105 days of gestation. Because of the late stage at which it is reliable, it is of little value in the early identification of non-pregnant animals. Estrone sulfate can be detected in sheep plasma from 70 days after conception, whereas in does it can be detected 40 to 50 days post-breeding.[98,100] Estrone sulfate concentrations measured in the milk of does using an enzyme-linked immunosorbent assay (ELISA) test were able to diagnose pregnancy with an accuracy of 82% and non-pregnancy with an accuracy of 83%.[101] Unfortunately, a positive ES test may or may not indicate the presence of a viable fetus.[102]

Progesterone assay

Measurement of concentrations of progesterone in blood and milk is one method for diagnosing pregnancy. The CL is the principal source of progesterone in the cyclic ruminants and is required to maintain early pregnancy.[103-105] Concentrations of progesterone are also elevated during the luteal phase of non-fertile estrous cycles and thus are not specific indicators of pregnancy. However, determining progesterone concentrations in milk or blood after breeding has been somewhat successful. If ruminants are pregnant, the CL persists and progesterone concentrations remain high while in non-pregnant animals, luteolysis occurs at the end of a non-fertile cycle and progesterone concentrations are low.[106,107]

Concentrations of plasma progesterone are determined 18 days post-breeding in ewes and 21 to 24 days post-breeding in does and cattle.[104,108,109] Concentrations of plasma progesterone measured in ewes on day 18 post-breeding showed that all females diagnosed non-pregnant did not lamb, while 84% of those diagnosed pregnant did lamb and is similar in does as accuracy of diagnosing pregnancy and non-pregnancy in does by determining serum progesterone concentrations was 86% and 100%, respectively.[110]

Progesterone concentrations in milk generally reflect plasma concentrations; however, concentrations of progesterone in milk are much higher.[101,106] Milk progesterone concentrations are above 10 ng per ml from 22 and 26 days after breeding in does classified as positive for pregnancy. Like plasma progesterone concentrations, an accuracy of 86% for detecting pregnancy and 100% for detecting non-pregnancy was reported in does.[111] Unfortunately milk progesterone concentration varies from day to day and also with the type of milk sample obtained.[112] Analysis of milk progesterone has been used as a method of pregnancy diagnosis in cattle at 20 to 23 days with accuracy similar to rectal palpation.[113] Measurement of concentrations of progesterone in milk is reasonably accurate for identification of non-pregnant cows (94%) but is not sufficiently accurate (77%) for recognition of pregnant cows.[114] Consequently, serum and plasma concentrations of progesterone tend to be more reliable and accurate predictors of pregnancy than concentrations determined from milk.

Overall progesterone testing accurately predicts non-pregnancy but is only a fair test for diagnosing pregnancy. Increased progesterone concentrations only indicate the presence of a functional CL and there are several conditions that may extend the luteal life and result in false positive results.[8,107] A benefit of determining circulating or milk progesterone concentrations as an indicator of extended CL life–and possibly pregnancy–is that false negatives for pregnancy almost never occur. However, false positives for pregnancy diagnosis are common and measurement of progesterone concentrations in blood and milk requires expensive technology.

Early pregnancy factor/early conception factor

Early pregnancy factor is a glycoprotein that is produced as a result of fertilization and can be detected in serum or milk of pregnant cattle as early as one to two days after insemination. Early pregnancy factor is present in plasma within hours of conception and concentrations fall below detectable levels soon after death or removal of the embryo.[115-117] Initially the assay for detecting early pregnancy factor was sensitive but time-consuming and not suitable for routine use.[118] More recently, the early conception factor test was marketed as a "cow side" immunoassay capable of diagnosing the non-pregnant cow within 12 to 48 h after ovulation.[119] Unfortunately, this test has been reported as inaccurate in cattle and horses.[119-123]

Pregnancy associated glycoprotein

The pregnancy-associated glycoproteins (PAGs) are abundantly expressed in the outer cell layer of the placenta of ruminants from the time the placenta attaches until parturition.[124] Pregnancy-associated glycoproteins

are separated into two groups (PAG-1 and PAG-2) and at least 21 bovine PAGs have been identified.[124,125] In cattle, some PAGs from the PAG-1 subgroup become detectable in the maternal circulation beginning at approximately the time of implantation (day 25). Concentrations of these glycoproteins steadily increased throughout pregnancy, peaking just before parturition.

The most widely recognized work with PAGs has been the development and commercial application of pregnancy-specific protein B (PSP-B) as a biochemical marker of pregnancy; BioPryn® (BioTracking, LLC, Moscow, ID) is a commercially available ELISA designed to detect PSP-B.[126-129] Immunoassays for PAGs other than PSP-B that are likely based on similar antigens have been developed.[130-135] Mean PAG concentrations in cattle begin to increase from days 15 to 35, but variation in serum PAG concentrations among cows precludes their use as a reliable indicator of pregnancy until days 26 to 30.[136,137] The first report using specific antisera for detecting pregnancy-associated glycoproteins secreted by the binucleate cells of the trophectoderm into the maternal plasma, allowed for the accurate discrimination between pregnant and non-pregnant goats from 21 days after breeding.[138] Subsequent study indicated PAG determination was highly accurate (≥ 99%) on days 24 and 26 after mating in goats.[68] Detection of PAG's has also been used in sheep to accurately detect pregnancy. Pregnancy diagnosis by assay of PSP-B in does and ewes is also available commercially (BioPRYN®) and samples can be shipped to several different affiliated laboratories. According to the manufacturer's recommendation serum samples should be taken from does and ewes 30 and 22 days or more after breeding, respectively.[126]

Variations in inter-assay agreement of PAG occur and may be due to variations in the antiserum utilized. In two studies, one designed to determine factors affecting plasma PAG concentrations in pregnant high-producing dairy cows and the other to assess the predictive importance of maternal PAG concentrations in recipients carrying somatic clones to pregnancy outcome, PAG concentrations varied with the assay and more specifically the primary antibody being used.[139,140] It is likely that the different polyclonal antisera used in radioimmunoassay (RIA) systems to measure PAG recognize other PAG molecules, and because more than 100 genes encode various PAG molecules in ruminant placenta, varying affinities would explain the inconsistent or varying inter-assay results.[141] Assays utilizing heterologous PAG RIA systems may be more sensitive and demonstrate higher PAG concentrations, improving ability to detect early pregnancy.[142] Sensitivity and specificity were improved when a monoclonal antibody was used to detect PAG.[131] The monoclonal antibody detected only a few PAG-1 family members belonging to the binucleate trophoblasts cell-specific group; use of the monoclonal antibody permitted pregnancy to be detected in all animals by 28 days after artificial insemination.

Persistence of PAG concentrations in the postpartum period or after pregnancy loss is one shortcoming of pregnancy determination based on PAG. Managers and practitioners must be aware of this limitation of current testing technology. The proteins reach a maximum concentration in plasma at approximately the time of parturition; with their long half-life, they remain in circulation for two to three months after calving.[133,137,143,144] When using plasma PAG for pregnancy diagnosis, the concentrations expected at various times following conception and previous parturition must be taken into account.[137, 145] The current manufacturer's recommendation is that serum samples be taken from cattle 90 days or more after parturition and 30 days post-breeding.[126] Cows less than two months postpartum may have PAG concentrations above the pregnancy/open cut-off value, even if they had not been inseminated. An important consideration is that PAG detected by monoclonal antibodies have a relatively shorter half-life, averaging only 4.3 days during the post-calving period, and PAG detected with monoclonal antibodies were below pregnancy threshold concentrations by week eight postpartum and 2.7-7.0 days after induction of embryonic mortality.[131,146,147]

More recently a serum PAG-based, rapid ELISA pregnancy test for cattle has been described.[148] The advantages of the rapid ELISA PAG test are: 1) that the test can be performed on the farm or at a nearby facility in approximately 90 min and requires only a simple thermostatically controlled water bath, 2) the diagnosis of pregnant or non-pregnant can be made by a subjective visual assessment of blue color with a high degree of accuracy (sensitivity and negative predictive value approached 100% and the specificity and positive predictive value were greater than 92%), 3) because of the relatively short half-life of the PAG detected by the assay, pregnancy detection with the rapid ELISA test could be performed as early as 50 days postpartum, and 4) the rapid ELISA test was highly accurate (95%) when used as early as 25 to 29 days after insemination.[148] Lastly, an ELISA for measuring PAG in milk has also been tested.[149] Pregnancy could reliably be diagnosed from day 28 onwards by testing serum and from day 150 onwards by testing milk.[149] Thus, PAG determination in milk is possible, but, due to the low concentrations, a more sensitive assay is needed before this test has practical relevance.

Interferon-stimulated gene 15

Trophoblast cells of bovine, caprine, and ovine embryo secrete interferon tau (IFN-τ). This substance is responsible for the maternal recognition of pregnancy, and for preventing the release of prostaglandin $F_2\alpha$,

regression of the CL, and the subsequent return to estrus. In the absence of a viable embryo, there will be no or insufficient INF-τ secretion, resulting in normal luteolysis and a return to estrus. Interferon tau also induces synthesis and secretion of interferon-stimulated gene 15 (ISG15) in the uterus of cattle.[150-154] Subsequently ISG15 is released by the endometrium when IFN-τ is released from the conceptus and ISG15 expression in peripheral white blood cells was greater in pregnant cows than in non-pregnant cows 18 days after insemination.[150-152,155] Identification of non-pregnant cows on days 18 to 20 after first insemination would facilitate a second insemination of non-pregnant cows approximately 10 days earlier than waiting to use current imaging and/or chemical detection techniques.[156] Prediction of non-pregnant cows based on low ISG15 was 100% accurate when examining serial collections (days 15 to 32 after insemination).[155] However, low ISG15 mRNA was 89% accurate in correctly predicting non-pregnant cows when examined on a single day, day 18, following insemination.[155] In the same study, progesterone on a single day, day 18, had a negative predictive value of 100%.[155] This is an important practical issue, since tests with a high negative predictive value will avoid inadvertent treatment of pregnant females with luteolytic agents and reduce the chances of iatrogenic abortions.

Table 1. Guide to transrectal palpation of cattle for pregnancy diagnosis (modified and used here with permission courtesy of R.L. Carson).

Days*	Amniotic Vesicle Size†		Fetal Size†		Fetal Membrane Slip†,¶	Placentomes†	Conceptus Location	Fremitus**
	Relative	Finger Width‡	Relative	Crown-To-Nose§				
35	Pea	½			Ipsilateral		Pelvis	
42	Grape	1			Ipsilateral		Pelvis	
48	Plum	2			Bilateral		Pelvis	
53	Bantam Egg	3			Bilateral		Pelvis	
58	Grade A Egg	4			Bilateral		Pelvis	
60	Duck Egg	Palm			Bilateral		Pelvis	
65		Hand	Mouse	1	Bilateral		Pelvis	
75			Rat	2	Bilateral	Pea	Pelvis	
90			Rat	3	Bilateral	Dime	Pelvis	
105			Cat	4		Nickel	Abdomen	
120			Cat	Hand		Quarter	Abdomen	Ipsilateral
150			Beagle			Fifty Cent	Abdomen	Ipsilateral
180			Brittany			Fifty Cent	Abdomen	Ipsilateral
210			Pointer			Fifty Cent	Pelvis	Bilateral
240			Doberman			Fifty Cent	Pelvis	Bilateral
270			German Shepherd			Fifty Cent	Pelvis	Bilateral

*Day of gestation.
†Positive/Cardinal signs of pregnancy
‡Finger approximately 1.5 to 2.0 cm wide.
§Finger and hand widths.
¶Palpation of the chorioallantoic membrane (fetal membrane slip) will initially be detected in the gravid horn but later in gestation is detected in the non-gravid horn as well
**Depending on the stage of pregnancy there will be fremitus in the middle uterine artery ipsilateral to the gravid horn or bilateral.

References

1. Zemjanis R: Diagnostic and therapeutic techniques in animal reproduction. Baltimore: Williams and Wilkins; 1971.
2. Rorie RW, Bilby TR, Lester TD: Application of electronic estrus detection technologies to reproductive management of cattle. Theriogenology 2002;57:137-148.
3. Watt BR, Anderson GA, Campbell IP: A comparison of six methods used for detecting pregnancy in sheep. Aust Vet J 1984;61:377-382.
4. Senger PL: The estrus detection problem: new concepts, technologies, and possibilities. J Dairy Sci 1994;77:2745-2753.
5. Dransfield MB, Nebel RL, Pearson RE, et al: Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J Dairy Sci 1998;81:1874-1882.
6. Sood P, Nanda AS: Effect of lameness on estrous behavior in crossbred cows. Theriogenology 2006;66:1375-1380.
7. Hesselink JW: Incidence of hydrometra in dairy goats. Vet Rec 1993;132:110-112.
8. Ishwar AK: Pregnancy diagnosis in sheep and goats-a review. Small Rumin Res 1995;17:37-44.
9. Richardson C: Pregnancy diagnosis in the ewe: a review. Vet Rec 1972;90:264-275.
10. Fowler PA, Knight CH, Foster MA: Omitting the dry period between lactations does not reduce subsequent milk-production in goats. J Dairy Res 1991;58:13-19.
11. Goel AK, Agrawal KP: A review of pregnancy diagnosis techniques in sheep and goats. Small Rumin Res 1992;9:255-264.
12. Aswad A, Abdou MS, Al-Bayaty F, et al: The validity of the "ultra-sonic method" for pregnancy diagnosis in ewes and goats. Zentralbl Veterinarmed A 1976;23:467-474.
13. Pratt MS, Hopkins PS: Diagnosis of pregnancy in sheep by abdominal palpation. Aust Vet J 1975;51:378-380.
14. Hulet CV: Rectal-abdominal palpation technique for diagnosing pregnancy in ewe. J Anim Sci 1972;35:814-819.

15. Ott RS, Braun WF, Lock TF, et al: A comparison of intrarectal Doppler and rectal abdominal palpation for pregnancy testing in goats. J Am Vet Med Assoc 1981;178:730-731.
16. Shelton M: Reproduction and breeding of goats. J Dairy Sci 1978;61:994-1010.
17. Tyrrell RN, Plant JW: Rectal damage in ewes following pregnancy diagnosis by rectal-abdominal palpation. J Anim Sci 1979;48:348-350.
18. Memon MA, Ott RS: Methods of pregnancy diagnosis in sheep and goats. Cornell Vet 1980;70:226-31.
19. Lamond DR: Diagnosis of early pregnancy in the ewe. Aust Vet J 1963;39:192-195.
20. Hulet CV, Foote WC: A rapid technique for observing reproductive tract of living ewes. J Anim Sci 1968;27:142-145.
21. Smith MC: Caprine reproduction. In: Morrow DA, editor. Current therapy in theriogenology. Philadelphia: Saunders; 1980. p. 975-977.
22. Cutten IN: Immediate and long term effects of postmating laparotomy on the lambing performance of Merino ewes. Aust Soc Anim Prod 1979. p. 388.
23. Richards C: Pregnancy diagnosis in the ewe: a review. Vet Rec 1972;90:264-275.
24. Hashimoto H: Diagnosis of pregnancy in the ewe. Can J Comp Med Vet Sci 1961;25:51-53.
25. Blair GW, Folley SJ, Malpress FH, et al: Variations in certain properties of bovine cervical mucus during the oestrous cycle. Biochem J 1941;35:1039-1049.
26. Fricke PM: Scanning the future - ultrasonography as a reproductive management tool for dairy cattle. J Dairy Sci 2002;85:1918-1926.
27. Griffin PG, Ginther OJ: Research applications of ultrasonic-imaging in reproductive biology. J Anim Sci 1992;70:953-972.
28. Kelton DF, Leslie KE, Etherington WG, et al: Accuracy of rectal palpation and of a rapid milk progesterone enzyme-immunoassay for determining the presence of a functional corpus luteum in subestrous dairy cows. Can Vet J 1991;32:286-291.
29. Bicalho RC, Galvao KN, Guard CL, et al: Optimizing the accuracy of detecting a functional corpus luteum in dairy cows. Theriogenology 2008;70:199-207.
30. Kelton DF: Accuracy of palpation of bovine corpora lutea. Cornell Vet 1989;79:301-305.
31. Cowie AT: Pregnancy diagnosis tests: a review. Shinfield (UK): Commonwealth Agricultural Bureaux; 1948. p. 11-17.
32. Sheldon M, Noakes D: Pregnancy diagnosis in cattle. In Pract 2002;24:310-317.
33. Wisnicky W, Casida LE: A manual method for the diagnosis of pregnancy in cattle. J Am Vet Med Assoc 1948;113:451-452.
34. Roberts SJ: Veterinary obstetrics and genital disease, 2nd ed. Woodstock (VT): Published by the author, 1971.
35. Ball L, Carroll EJ: Induction of fetal death in cattle by manual rupture of amniotic vesicle. J Am Vet Med Assoc 1963;142:373-374.
36. Rowson LEA, Lawson RAS, Moor RM: Production of twins in cattle by egg transfer. J Reprod Fertil 1971;25:261-268.
37. Dawson FLM: Methods for early termination of pregnancy in cow. Vet Rec 1974;94:542-548.
38. Parmigiani E, Ball L, Lefever D, et al: Elective termination of pregnancy in cattle by manual abortion. Theriogenology 1978;10:283-290.
39. Abbitt B, Ball L, Kitto GP, et al: Effect of 3 methods of palpation for pregnancy diagnosis per rectum on embryonic and fetal attrition in cows. J Am Vet Med Assoc 1978;173:973-977.
40. Paisley LG, Mickelsen WD, Frost OL: Survey of incidence of prenatal mortality in cattle following pregnancy diagnosis by rectal palpation. Theriogenology 1978;9:481-491.
41. Franco OJ, Drost M, Thatcher MJ, et al: Fetal survival in the cow after pregnancy diagnosis by palpation per rectum. Theriogenology 1987;27:631-644.
42. White ME, Lafaunce N, Mohammed HO: Calving outcomes for cows diagnosed pregnant or nonpregnant by per rectum examination at various intervals after insemination. Can Vet J 1989;30:867-870.
43. Vaillancourt D, Bierschwal CJ, Ogwu D, et al: Correlation between pregnancy diagnosis by membrane slip and embryonic mortality. J Am Vet Med Assoc 1979;175:466-468.
44. Thurmond MC, Picanso JP: Fetal loss associated with palpation per rectum to diagnose pregnancy in cows. J Am Vet Med Assoc 1993;203:432-435.
45. Thompson JA, Marsh WE, Calvin JA, et al: Pregnancy attrition associated with pregnancy testing by rectal palpation. J Dairy Sci 1994;77:3382-3387.
46. Brenner J, Orgad U: Epidemiological investigations of an outbreak of intestinal atresia in two Israeli dairy herds. J Vet Med Sci 2003;65:141-143.
47. Constable PD, Shanks RD, Huhn J, et al: Evaluation of breed as a risk factor for atresia coli in cattle. Theriogenology 1997;48:775-790.
48. Ness H, Leopold G, Muller W: Genesis of congenital ileus in calf (artresia coli and artresia jejuni). Monatsh Veterinarmed 1982;37:89-92.
49. Romano JE, Thompson JA, Kraemer DC, et al: Early pregnancy diagnosis by palpation per rectum: influence on embryo/fetal viability in dairy cattle. Theriogenology 2007;67:486-493.
50. Benzie D: X-ray diagnosis of pregnancy in ewes. Br Vet J 1951;107:3-6.
51. Ford E, Clark J, Gallup A: The detection of foetal numbers in sheep by means of x-rays. Vet Rec 1963;75:958-960.
52. Barker CA, Cawley AJ: Radiographic detection of fetal numbers in goats. Can Vet J 1967;8:59-61.
53. Matsas D: Pregnancy diagnosis in goats. In: Youngquist RS, Threlfall WR, editors. Current therapy in large animal theriogenology. 2nd ed. St. Louis: Saunders; 2007. p. 547-554.
54. Baxter SJ, Ward WR: Incidence of fetal loss in dairy cattle after pregnancy diagnosis using an ultrasound scanner. Vet Rec 1997;140:287-288.
55. Wurst AK, Dixon AB, Inskeep EK: Lack of effect of transrectal ultrasonography with restraint on lambing rate and prolificacy in ewes. Theriogenology 2007;68:1012-1016.
56. Bretzlaff KN: Development of hydrometra in a ewe flock after ultrasonography for determination of pregnancy. J Am Vet Med Assoc 1993;203:122-125.
57. Lindahl IL: Pregnancy diagnosis in dairy goats using ultrasonic Doppler instruments. J Dairy Sci 1969;52:529-30.
58. Haibel GK: Use of ultrasonography in reproductive management of sheep and goat herds. Vet Clin North Am Food Anim Pract 1990;6:597-613.

59. Ishwar AK: Pregnancy diagnosis in sheep and goats-a review. Small Rumin Res 1995;17:37-44.
60. Fraser AF, Nagaratnam V, Callicott RB: The comprehensive use of Doppler ultrasound in farm animal reproduction. Vet Rec 1971;88:202-205.
61. Pierson RA, Ginther OJ: Ultrasonography for detection of pregnancy and study of embryonic development in heifers. Theriogenology 1984;22:225-233.
62. Kastelic JP, Curran S, Ginther OJ: Accuracy of ultrasonography for pregnancy diagnosis on days 10 to 22 in heifers. Theriogenology. 1989; 31:813-820.
63. Romano JE, Thompson JA, Forrest DW, et al: Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. Theriogenology 2006;66:1034-1041.
64. Gaja A, Kubota C, Kojima T: Early pregnancy diagnosis in Japanese black cows using a novel transrectal ultrasonography protocol. Vet Rec 2009;164:659-660.
65. Hunnam JC, Parkinson TJ, Lopez-Villalobos N, et al: Comparison of transcutaneous ultrasound over the right flank with transrectal ultrasound for pregnancy diagnosis in the dairy cow. Aust Vet J 2009;87:318-322.
66. Buczinski S: Ultrasonographic assessment of late term pregnancy in cattle. Vet Clin North Am Food Anim Pract 2009;25:753-765.
67. Schrick FN, Inskeep EK: Determination of early-pregnancy in ewes utilizing transrectal ultrasonography. Theriogenology 1993;40:295-306.
68. Gonzalez F, Cabrera F, Batista M, et al: A comparison of diagnosis of pregnancy in the goat via transrectal ultrasound scanning, progesterone, and pregnancy-associated glycoprotein assays. Theriogenology 2004;62:1108-1115.
69. Garcia A, Neary MK, Kelly GR, et al: Accuracy of ultrasonography in early-pregnancy diagnosis in the ewe. Theriogenology 1993;39:847-861.
70. Bretzlaff KN, Bretzlaff J, Edwards J, et al: Ultrasonographic determination of pregnancy in small ruminants. Vet Med 1993;88:12-24.
71. Buckrell BC: Applications of ultrasongoraphy in reproduction in sheep and goats. Theriogenology 1988;29:71-84.
72. Gearhart MA, Wingfield WE, Knight AP, et al. Real-time ultrasonography for determining pregnancy status and viable fetal numbers in ewes. Theriogenology 1988;30:323-337.
73. Bazer F, Cunningham W, Marsh D: Pregnancy diagnosis. In: Youngquist RS, Threlfall WR, editors. Current therapy in large animal theriogenology. 2nd ed. St. Louis: Saunders; 2007. p. 661-666.
74. Karen A, Szabados K, Reiczigel J, et al: Accuracy of transrectal ultrasonography for determination of pregnancy in sheep: effect of fasting and handling of the animals. Theriogenology 2004;61:1291-1298.
75. Fowler DG, Wilkins JF: Diagnosis of pregnancy and number of fetuses in sheep by real-time ultrasonic-imaging. 1. Effects of number of fetuses, stage of gestation, operator and breed of ewe on accuracy of diagnosis. Livest Prod Sci 1984;11:437-450.
76. White IR, Russel AJ, Fowler DG: Real-time ultrasonic scanning in the diagnosis of pregnancy and the determination of fetal numbers in sheep. Vet Rec 1984;115:140-143.
77. Muller E, Wittkowski G: Visualization of male and female characteristics of bovine fetuses by real-time ultrasonics. Theriogenology 1986;25:571-574.
78. Curran S, Kastelic JP, Ginther OJ: Determining sex of the bovine fetus by ultrasonic assessment of the relative location of the genital tubercle. Anim Reprod Sci 1989;19:217-227.
79. Coubrough CA, Castell MC: Fetal sex determination by ultrasonically locating the genital tubercle in ewes. Theriogenology 1998;50:263-267.
80. Ali A: Effect of gestational age and fetal position on the possibility and accuracy of ultrasonographic fetal gender determination in dairy cattle. Reprod Domest Anim 2004;39:190-194.
81. Amer HA: Ultrasonographic assessment of early pregnancy diagnosis, fetometry and sex determination in goats. Anim Reprod Sci 2010;117:226-231.
82. Santos MH, Rabelo MC, Guido SI, et al. Determination of the genital tubercle migration period in Morada Nova sheep foetuses by ultrasonography. Reprod Domest Anim 2007;42:214-217.
83. Burstel D, Meinecke-Tillman S, Meinecke B: Ultrasonographic diagnosis of fetal sex in small ruminants bearing multiple fetuses. Vet Rec 2002;151:635-636.
84. White IR, Russel AJ, Wright IA, et al: Real-time ultrasonic scanning in the diagnosis of pregnancy and the estimation of gestational age in cattle. Vet Rec 1985;117:5-8.
85. Kahn W: Sonographic fetometry in the bovine. Theriogenology 1989;31:1105-1121.
86. Suguna K, Mehrotra S, Agarwal SK, et al: Early pregnancy diagnosis and embryonic and fetal development using real time B mode ultrasound in goats. Small Rumin Res 2008;80:80-86.
87. Martinez MF, Bosch P, Bosch RA: Determination of early pregnancy and embryonic growth in goats by transrectal ultrasound scanning. Theriogenology 1998;49:1555-1565.
88. Hunnam JC, Parkinson TJ, Lopez-Villalobos N, et al: Association between gestational age and bovine fetal characteristics measured by transcutaneous ultrasound over the right flank of the dairy cow. Aust Vet J 2009;87:379-383.
89. Greenwood PL, Slepetis RM, McPhee MJ, et al: Prediction of stage of pregnancy in prolific sheep using ultrasound measurement of fetal bones. Reprod Fertil Dev 2002;14:7-13.
90. Doize F, Vaillancourt D, Carabin H, et al: Determination of gestational age in sheep and goats using transrectal ultrasonographic measurement of placentomes. Theriogenology 1997;48:449-460.
91. Ginther OJ: Ultrasonic imaging and animal reproduction. Cross Plains (WI): Equiservices Publishing; 1995.
92. Buczinski SM, Fecteau G, Lefebvre RC, et al: Fetal well-being assessment in bovine near-term gestations: current knowledge and future perspectives arising from comparative medicine. Can Vet J 2007;48:178-183.
93. Hanzen C, Pieterse M, Scenczi O, et al: Relative accuracy of the identification of ovarian structures in the cow by ultrasonography and palpation per rectum. Vet J 2000;159:161-170.
94. Honnens A, Voss C, Herzog K, et al: Uterine blood flow during the first 3 weeks of pregnancy in dairy cows. Theriogenology 2008;70:1048-1056.
95. Utt MD, Johnson GL, Beal WE: The evaluation of corpus luteum blood flow using color-flow Doppler ultrasound for early pregnancy diagnosis in bovine embryo recipients. Theriogenology 2009;71:707-715.

96. Dobson H, Rowan TG, Kippax IS, et al: Assessment of fetal number, and fetal and placental viability throughout pregnancy in cattle. Theriogenology 1993;40:411-425.
97. McArthur CP, Geary A: Field evaluation of a pregnancy immunoassay for the detection of oestrone sulphate in goats. J Endocrinol 1986;110:133-136.
98. Tsang CPW: Plasma-levels of estrone sulfate, free estrogens and progesterone in pregnant ewe throughout gestation. Theriogenology 1978;10:97-110.
99. Robertson HA, King GJ: Conjugated and unconjugated oestrogens in fetal and maternal fluids of the cow throughout pregnancy. J Reprod Fertil 1979;55:463-470.
100. Refsal KR, Marteniuk JV, Williams CSF, et al: Concentrations of estrone sulfate in peripheral serum of pregnant goats-relationships with gestation length, fetal number and the occurrence of fetal death in utero. Theriogenology 1991;36:449-461.
101. Murray RD, Newstead R: Determination of steroid hormones in goats' milk and plasma as an aid to pregnancy diagnosis using an ELISA. Vet Rec 1988;122:158-161.
102. Jonker FH: Fetal death: comparative aspects in large domestic animals. Anim Reprod Sci 2004;82-83:415-430.
103. Davis JS, Rueda BR: The corpus luteum: an ovarian structure with maternal instincts and suicidal tendencies. Front Biosci 2002;7:d1949-1978.
104. Spencer TE, Bazer FW: Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. Front Biosci 2002;7:d1879-1898.
105. Niswender GD, Juengel JL, Silva PJ, et al: Mechanisms controlling the function and life span of the corpus luteum. Physiol Rev 2000;80:1-29.
106. de Montigny G, Millerioux P, Jeanguyot N, et al: Milk fat progesterone concentrations in goats and early pregnancy diagnosis. Theriogenology 1982;17:423-431.
107. Fleming SA, Van Camp SD, Chapin HM: Serum progesterone determination as an aid for pregnancy diagnosis in goats bred out of season. Can Vet J 1990;31:104-107.
108. Corteel JM, Gonzalez C, Nunes JF: Research and development in the control of reproduction. Third Int Conf Goat Prod Disease 1982. p. 584-601.
109. McPhee IM, Tiberghien MP: Assessment of pregnancy in sheep by analysis of plasma progesterone using an amplified enzyme immunoassay technique. Vet Rec 1987;121:63-65.
110. Thibier J, Jeanguyot N, DeMontigny G: Accuacy of early-pregnancy diatnosis in goats based on plasma and milk progesterone concentrations. Int Goat Sheep Res 1982;2:1-6.
111. Holdsworth RJ, Davies J: Measurement of progesterone in goat's milk: an early pregnancy test. Vet Rec 1979;105:535.
112. Bretzlaff KN, Elmore RG, Nuti LC: Use of an enzyme immunoassay to determine concentrations of progesterone in caprine plasma and milk. J Am Vet Med Assoc 1989;194:664-668.
113. Hoffmann B, Hamburger R, Gunzler D, et al: Determination of progesterone in milk applied for pregnancy diagnosis in the cow. Theriogenology 1974;2:21-28.
114. Gowan EW, Etches RJ, Bryden C, et al: Factors affecting accuracy of pregnancy diagnosis in cattle. J Dairy Sci 1982;65:1294-1302.
115. Koch E, Morton H, Ellendorff F: Early pregnancy factor: biology and practical application. Br Vet J 1983;139:52-58.
116. Sakonju I, Enomoto S, Kamimura S, et al: Monitoring bovine embryo viability with early pregnancy factor. J Vet Med Sci 1993;55:271-274.
117. Fan XG, Zheng ZQ: A study of early pregnancy factor activity in preimplantation. Am J Reprod Immunol 1997;37:359-64.
118. Yoshioka K, Iwamura S, Kamomae H: Application of anti-bovine CD2 monoclonal antibody to the rosette inhibition test for detection of early pregnancy factor in cattle. J Vet Med Sci 1995;57:721-725.
119. Cordoba MC, Sartori R, Fricke PM: Assessment of a commercially available early conception factor (ECF) test for determining pregnancy status of dairy cattle. J Dairy Sci 2001;84:1884-1889.
120. Gandy B, Tucker W, Ryan P, et al: Evaluation of the early conception factor (ECF™) test for the detection of nonpregnancy in dairy cattle. Theriogenology 2001;56:637-647.
121. Ambrose DJ, Radke B, Pitney PA, et al: Evaluation of early conception factor lateral flow test to determine nonpregnancy in dairy cattle. Can Vet J 2007;48:831-835.
122. Marino E, Threlfall WR, Schwarze RA: Early conception factor lateral flow assays for pregnancy in the mare. Theriogenology 2009;71:877-883.
123. Horteloup MP, Threlfall WR, Funk JA: The early conception factor (ECF) lateral flow assay for non-pregnancy determination in the mare. Theriogenology 2005;64:1061-1071.
124. Wooding FB, Roberts RM, Green JA: Light and electron microscope immunocytochemical studies of the distribution of pregnancy associated glycoproteins (PAGs) throughout pregnancy in the cow: possible functional implications. Placenta 2005;26:807-827.
125. Green JA, Xie S, Quan X, et al: Pregnancy-associated bovine and ovine glycoproteins exhibit spatially and temporally distinct expression patterns during pregnancy. Biol Reprod 2000;62:1624-1631.
126. Biotracking.com [homepage on the Internet]. Moscow (ID): Biotracking; c2007 [cited 2010 June 14]. Available from: http://www.biotracking.com.
127. Butler JE, Hamilton WC, Sasser RG, et al: Detection and partial characterization of two bovine pregnancy-specific proteins. Biol Reprod 1982;26:925-933.
128. Sasser RG, Crock J, Ruder-Montgomery CA: Characteristics of pregnancy-specific protein B in cattle. J Reprod Fertil Suppl 1989;37:109-13.
129. Sasser RG, Ruder CA, Ivani KA, et al: Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. Biol Reprod 1986;35:936-942.
130. Xie SC, Low BG, Nagel RJ, et al: Identification of the major pregnancy-specific antigens of cattle and sheep as inactive members of the aspartic proteinase family. Proc Natl Acad Sci U S A 1991;88:10247-10251.
131. Green JA, Parks TE, Avalle MP, et al: The establishment of an ELISA for the detection of pregnancy-associated glycoproteins (PAGs) in the serum of pregnant cows and heifers. Theriogenology 2005;63:1481-1503.
132. Zoli AP, Beckers JF, Wouters-Ballman P, et al: Purification and characterization of a bovine pregnancy-associated glycoprotein. Biol Reprod 1991;45:1-10.

133. Mialon MM, Camous S, Renand G, et al: Peripheral concentrations of a 60-kDa pregnancy serum protein during gestation and after calving and in relationship to embryonic mortality in cattle. Reprod Nutr Dev 1993;33:269-282.
134. Zoli AP, Guilbault LA, Delahaut P, et al: Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum - its application for pregnancy diagnosis. Biol Reprod 1992;46:83-92.
135. Lynch RA, Alexander BM, Sasser RG: The cloning and expression of the pregnancy-specific protein B (bPSPB) gene. Biol Reprod 1992;46(Suppl 1):72 [abstract].
136. Humblot P: Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology 2001;56:1417-1433.
137. Zoli AP, Guilbault LA, Delahaut P, et al: Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: its application for pregnancy diagnosis. Biol Reprod 1992;46:83-92.
138. Gonzalez F, Sulon J, Garbayo JM, et al: Early pregnancy diagnosis in goats by determination of pregnancy-associated glycoprotein concentrations in plasma samples. Theriogenology 1999;52:717-725.
139. Lopez-Gatius F, Garbayo JM, Santolaria P, et al: Milk production correlates negatively with plasma levels of pregnancy-associated glycoprotein (PAG) during the early fetal period in high producing dairy cows with live fetuses. Domest Anim Endocrinol 2007;32:29-42.
140. Chavatte-Palmer P, de Sousa N, Laigre P, et al: Ultrasound fetal measurements and pregnancy associated glycoprotein secretion in early pregnancy in cattle recipients carrying somatic clones. Theriogenology 2006;66:829-840.
141. Xie S, Green J, Bixby JB, et al: The diversity and evolutionary relationships of the pregnancy-associated glycoproteins, an aspartic proteinase subfamily consisting of many trophoblast-expressed genes. Proc Natl Acad Sci U S A 1997;94:12809-12816.
142. Perenyi ZS, Szenci O, Sulon J, et al: Comparison of the ability of three radioimmunoassay to detect pregnancy-associated glycoproteins in bovine plasma. Reprod Domest Anim 2002;37:100-104.
143. Patel OV, Sulon J, Beckers JF, et al: Plasma bovine pregnancy-associated glycoprotein concentrations throughout gestation in relationship to fetal number in the cow. Eur J Endocrinol 1997;137:423-428.
144. de Sousa NM, Zongo M, Pitala W, et al: Pregnancy-associated glycoprotein concentrations during pregnancy and the postpartum period in Azawak Zebu cattle. Theriogenology 2003;59:1131-1142.
145. Szenci O, Beckers JF, Humblot P, et al: Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology 1998;50:77- 88.
146. Semambo DK, Eckersall PD, Sasser RG,et al: Pregnancy-specific protein B and progesterone in monitoring viability of the embryo in early pregnancy in the cow after experimental infection with Actinomyces pyogenes. Theriogenology 1992;37:741-748.
147. Szenci O, Beckers JF, Sulon J, et al: Effect of induction of late embryonic mortality on plasma profiles of pregnancy associated glycoprotein 1 in heifers. Vet J 2003;165:307-313.
148. Green JC, Volkmann DH, Poock SE, et al: Technical note: a rapid enzyme-linked immunosorbent assay blood test for pregnancy in dairy and beef cattle. J Dairy Sci 2009;92:3819-3824.
149. Friedrich M, Holtz W: Establishment of an ELISA for measuring bovine pregnancy-associated glycoprotein in serum or milk and its application for early pregnancy detection. Reprod Domest Anim 2010;45:142-146.
150. Austin KJ, Ward SK, Teixeira MG, et al: Ubiquitin cross-reactive protein is released by the bovine uterus in response to interferon during early pregnancy. Biol Reprod 1996;54:600-606.
151. Hansen TR, Austin KJ, Johnson GA: Transient ubiquitin cross-reactive protein gene expression in the bovine endometrium. Endocrinology 1997;138:5079-5082.
152. Johnson GA, Austin KJ, Van Kirk EA, et al: Pregnancy and interferon-tau induce conjugation of bovine ubiquitin cross-reactive protein to cytosolic uterine proteins. Biol Reprod 1998;58:898-904.
153. Perry DJ, Austin KJ, Hansen TR: Cloning of interferon-stimulated gene 17: the promoter and nuclear proteins that regulate transcription. Mol Endocrinol 1999;13:1197-1206.
154. Thatcher WW, Guzeloglu A, Mattos R, et al: Uterine-conceptus interactions and reproductive failure in cattle. Theriogenology 2001;56:1435-1450.
155. Han HC, Austin KJ, Rempel LA, et al: Low blood ISG15 mRNA and progesterone levels are predictive of non-pregnant dairy cows. J Endocrinol 2006;191:505-512.
156. Lucy MC. Reproductive loss in high-producing dairy cattle: where will it end? J Dairy Sci 2001;84:1277-1293.

Update on heritable congenital defects in cattle

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Abstract

Inherited congenital anomalies are probably present in all breeds of cattle and propagated as a result of specific trait selection. In some breeds, the occurrence of inherited anomalies has become frequent, and economically important. Veterinarians, animal scientists, and cattle breeders should be aware of inherited defects, and be prepared to investigate and report animals exhibiting abnormal phenotypes. This review will describe the morphologic characteristics, mode of inheritance, breeding lines affected, and the availability of testing for selected (newly described) heritable bovine fetal abnormalities.

Keywords: Inherited, congenital, defects, cattle, bovine

Introduction

Genetic defects in cattle are being recognized at an increasing rate and genetic testing has changed cattle production–not only in terms of traits beneficial to production, but also in the ability to identify and manage harmful genetic defects. As selection concentrates the genetics of certain individuals, the potential for emergence of heritable anomalies increase. The surveillance of such disorders has become an important part of bovine health programs.

When a potentially heritable fetal abnormality is discounted as a randomly occurring "accident of gestation", the defect may not be deemed reportable, and appropriate samples may not be collected. Failure of identification or delay in detection of inherited congenital anomalies may allow further distribution of the mutated genetics. Obvious defects such as skeletal malformations, extensive soft tissue abnormalities, severe neurological disorders, and diseases of the skin are more likely to be recognized, whereas defects involving internal organs may be less obvious and more easily missed. Surveillance may be further compromised by the reluctance to report potentially heritable disorders, or the reluctance of breed associations to aggressively pursue potentially heritable disorders.

Several reviews on inherited disorders in cattle have been published[1-4] and a regularly updated electronic database, Online Mendelian Inheritance in Animals (OMIA), is available on the worldwide web (http://omia.angis.org.au/).[5] The purpose of this review is to discuss those heritable bovine fetal abnormalities recently described for which the mutation has been identified and a test is available.

Arthrogryposis multiplex

Arthrogryposis multiplex (AM; "curly calf syndrome") is a lethal autosomal recessive genetic defect that originated in Angus cattle. Beginning in 2008, researchers in collaboration with the American Angus Association investigated abnormal calves believed to fit the description of what was then called AM and commonly referred to as "curly calf syndrome" in Angus cattle. Within two months, researchers obtained samples and pedigrees from affected calves and their parents, the mutation was identified, the DNA test was developed and validated, and the status of over 700 bulls used for artificial insemination (AI) was determined.[6]

The genetic cause of AM was suspected when pedigree analysis of the original cases found that all affected calves trace on one or both sides of their pedigree to GAR Precision 1680. Later investigation revealed that those AM calves that did not trace to 1680 on both sides of the pedigree did trace to his dam 9J9 GAR 856 whose sire Rito 9J9 of B1567T26 has subsequently been determined to be a carrier of AM.[7]

Calves with AM are born dead or die shortly after birth. They are small for gestational age and have markedly diminished muscle mass. It appears that in AM an essential protein that allows communication between nerves and muscle tissue is absent, thus the calf (which fails to move *in utero*) is born with the joints of all four limbs fixed and the legs twisted. There are several characteristics of AM including arthrogryposis (fixed, twisted joints), kyphoscoliosis (twisted spine), and decreased muscling (Figure 1).[6]

The mutation is a deletion that involves three genes–one of these genes is involved in the development of nerve and muscle. Affected calves are missing ~23,000 base pairs. These missing base pairs result in complete loss of function of all three genes in homozygous calves.[7] A genetic test is available through several laboratories (AgriGenomics, Mansfield, IL; Igenity, Lincoln, NE; Pfizer Animal Genetics, Kalamazoo, MI; GeneSeek, Lincoln, NE; and MMI Genomics, Davis, CA) to determine if an animal carries the AM mutation.

Neuropathic hydrocephalus

Neuropathic hydrocephalus (NH) is a lethal autosomal recessive genetic defect of Angus cattle. At the same time that AM calves were being submitted, calves with hydrocephalus were also submitted. These calves were similar in description and pedigree to calves described by Dr. Denholm in Australia. Interestingly, calves submitted for both AM and NH generally had GAR Precision 1680 on both sides of the pedigree.

Affected NH calves are born near term and weigh 25-35 pounds at birth. The head is markedly enlarged (Figure 2A). The bones of the skull are malformed and appear as loosely organized bony plates that fall apart when the head is opened (Figure 2B). The cranium is filled with fluid and no recognizable brain tissue is evident. The spinal canal is also dilated and no observable spinal cord tissue is found.[8]

Neuropathic hydrocephalus is the consequence of single DNA base pair mutation on both alleles. The genetic mutation results in the abnormal function of an important protein that is involved in the development and maintenance of the central nervous system resulting in the NH syndrome.[8] The NH mutation likely originated with the bull GAR Precision 1680 as both his sire and dam test negative for NH.[8]

Nearly 10% of AI sires representing a broad cross section of registered Angus genetics were found to be carriers of NH.[9] Given the number of calves reported, this frequency appeared to be higher than expected. This phenomenon could be explained by a relatively high percentage (50% to 70%) of pregnancy wastage in NH embryos and/or fetuses. This is consistent with what is known about mutations in this gene for other species (i.e., complete disruption of this gene in mice results in 100% fetal mortality before the halfway point of gestation).[9] Genetic testing to determine if an animal carries the NH mutation is available through many of the same laboratories providing testing for AM (AgriGenomics, Igenity, Pfizer Animal Genetics, GeneSeek, and MMI Genomics).

Congenital contractural arachnodactyly

Congenital contractural arachnodactyly (CA), also known as "fawn calf syndrome", is a non-lethal autosomal recessive genetic defect of Angus cattle. Calves affected by CA are normally born alive and most can walk, suckle, and survive. The birthweight of CA calves is "normal". The phenotype is subtle and hence CA may not initially be recognized as a defect. Congenital contractural arachnodactyly is a developmental defect involving reduced elasticity of the connective tissue of muscles, first identified in Victoria, Australia in 1998 but now reported in many countries.[10] Although CA is a less severe disease than lethal genetic defects of Angus calves, without human intervention up to 20% of CA calves die soon after birth, simply because they are unable to stand and suckle.[10] To make the diagnosis in a newborn calf, it is necessary that all the following are observed:

- Congenital proximal limb contracture;
- Congenital distal limb hyperextension;
- Congenital kyphosis; and
- Significant post-natal improvement in these clinical signs as the calf grows and matures (Figure 3A, B).[10]

Since 2001, veterinarians and other scientists have been investigating CA in Angus cattle from Australia, with suspected cases in the USA and several other countries.[4] These investigations have included parental verification, pedigree analyses, physical examination, necropsy, and quantitative analysis of computer tomography scans of affected calves and their unaffected siblings. In 2004 Australian researchers demonstrated the genetic control of CA by embryo transfer matings of putative carrier sires to affected females. Those matings produced claves affected with the CA pathology in a proportion consistent with recessive inheritance. All Australian cases of CA identified to date have traced to Angus bulls (from the USA) whose semen was imported into Australia.

Researchers have identified the genetic defect that causes CA and have partially characterized the specific mutation responsible for CA as a deletion of at least 38,000 DNA base pairs that removes a significant portion of this gene severely compromising its function.[11] The complete sequence of the deleted DNA segment is not known making it currently impossible to develop a diagnostic test that is 100% accurate.[11] Until recently the breed associations (American Angus Association and Angus Australia) have avoided identifying any animal as a CA carrier because the current diagnostic test is less than 100% accurate.[12] However, some specifically identified animals have been named as either carriers or are "highly likely" to be carriers of the CA mutation by Angus Australia.[11] The current assay generates some false positives in a number of pedigrees creating a significant danger of misinterpretation of test results. The current test does allow an overall estimation of frequency of the CA mutation in the population. With more than 500 animals genotyped with several of the genetic markers for CA the maximum frequency of CA in the AI sire population is approximately 3 to 4%.[11]

Idiopathic epilepsy

Idiopathic epilepsy (IE) is a seizure disorder caused by an autosomal recessive genetic defect and is incompatible with life. The condition is predominately seen in horned Herefords, but can be seen in polled Herefords with horned animals in their pedigrees. Affected calves can have their first seizure any time from birth to several months of age and they have a "normal" phenotype when they are not affected by a seizure. Environmental stressors (heat, cold, weaning, etc.) can trigger the seizures and the episode can last from minutes to more than an hour.

There have been reports about a seizure disorder in Herefords for some time. Scientists began receiving reports of seizing calves and samples from them in 2003. Subsequently, tissue from *in vivo* fertilization using suspected carrier cows and bulls was used to identify the mutation.[7] Over 15,000 Hereford samples have been tested for IE. To date all carriers of IE trace to a single bull born in 1982, however DNA is not available to test this bull.

The mutation is more complicated than a single substitution or deletion. DNA base pairs are duplicated and deleted, with the result being an addition of 5 base pairs.[7] A DNA test is available through several laboratories (AgriGenomics, American Hereford Association, and Igenity) to determine if an animal carries the IE mutation.

Osteopetrosis

Osteopetrosis (OS; "marble bone") is a lethal autosomal recessive genetic defect previously identified in humans and a long list of animals. Cattle breeds known to be affected are Black and Red Angus, Hereford, Simmental, and Holstein. The defect was most recently reported in Red Angus cattle.[13] Calves affected with OS are born 10 to 30 days early. They usually have head abnormalities that consist of brachygnathia inferior, impacted molars, and a protruding tongue. The long bones are shorter than normal, the marrow cavities are filled with unreabsorbed bone (primary spongiosa), but are very fragile and can be easily broken.[13]

Samples from identified carriers were used to identify the mutation. The disease is caused by a deletion of a gene necessary for bone remodeling during development (SLC4A2). Genetic mutations that cause OS in Red Angus and Black Angus cattle are not the same. However, the mutation in Black Angus has not been identified. This could mean that the mutation in Black Angus changed or that they are two distinctly different mutations. Genetic testing is available through AgriGenomics, Pfizer Animal Health, MMI Genomics, and Igenity. However, the OS test is for the mutation in Red Angus only.

Summary

In recent years, there have been several defects identified that have had significant impact on specific cattle populations. The cooperation of molecular geneticists, veterinary pathologists, breeders, and breed associations has identified genetic disorders, characterized the pathology, determined the genetic mutations and then developed tests. In the very near future, it is likely that the genetic mutations in other heritable congenital defects of cattle will be identified through similar collaborative efforts.

Although the investigation of heritable bovine fetal anomalies has often been left to those in academia, specifically animal scientists and veterinary pathologists, without the assistance of private practitioners and producers, many of the currently recognized inherited disorders of cattle would have gone undiscovered. For any surveillance program to be successful, recognition of a potentially heritable defect is but the first step. The anomaly must be reported, appropriate samples collected and preserved, and pedigree information made available. Veterinarians in the field can play a pivotal role in discovery and surveillance by recognizing and reporting inherited defects of cattle.

References

1. Leipold HW, Huston K, Dennis SM: Bovine congenital defects. Adv Vet Sci Comp Med 1983; 27:197-271.
2. Liepold HW, Hiraga T, Dennis SM: Congenital defects of the vovine musculoskeletal system and joints. Vet Clin North Am Food Anim Pract 1993;9:93-104.
3. Huston K, Saperstein G, Steffen D, et al: Clinical, pathological and other visible traits loci except coat colour (category 2). In: Lauvergne JJ, Millar P, Dolling C, editors. Mendelian inheritance in cattle. Wageningen (The Netherlands): Wageningen Academic Publishers; 2000.
4. Whitlock BK, Kaiser L, Maxwell HS: Heritable bovine fetal abnormalities. Theriogenology 2008;70: 535-549.
5. Nicholas FW. Genetic databases: Online catalogues of inherited disorders. Rev Sci Tech 1998;17:346-350.
6. American Angus Association [homepage on the Internet]. St. Joseph (MO); c2010. Available from: http://www.angus.org/pub/am/aminfo.aspx).
7. Kaiser L: The gene gurus have been busy this decade: recessive defect, mutations and DNA-based tests [monograph on the Internet]. Available from: http://kaisercattle.com/pdf/Deadcows.pdf.
8. American Angus Association: Neuropathic hydrocephalus fact sheet, 2010. Available from: http://www.angus.org/pub/NH/NHFactSheet.pdf
9. Beever JE. Research update on hydrocephalus. Available from: http://www.angus.org/Pub/NH/Research_Update_Hydrocephalus.pdf

10. Denholm L. Congenital contractural arachnodactyly ('fawn calf syndrome') in Angus cattle. Primefact 1015, May 2010; Available from: http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0011/336944/Congenital-contractural-arachnodactyly-in-Angus-cattle.pdf.
11. Beever JE: Fawn calf syndrome. Available from: http://www.angus.org/Pub/FC/FC_Notice_052610.pdf.
12. Steffen D, Beever JE: Fawn calf syndrome update as of December 21, 2009. Available from: http://www.angus.org/Pub/FC/FC_Notice_122109.aspx.
13. Nietfeld J. Osteopetrosis in calves. Diagnostic Insights 1: 2;2007. Available from: http://www.vet.ksu.edu/depts/dmp/service/news/KSVDL_102.pdf.

Figure 1. Arthrogryposis multiplex in a Angus calf that was born dead. Notice the contracted forelimbs and extended hindlimbs. This photograph is courtesy of Dr. Robert L. Carson of Auburn University.

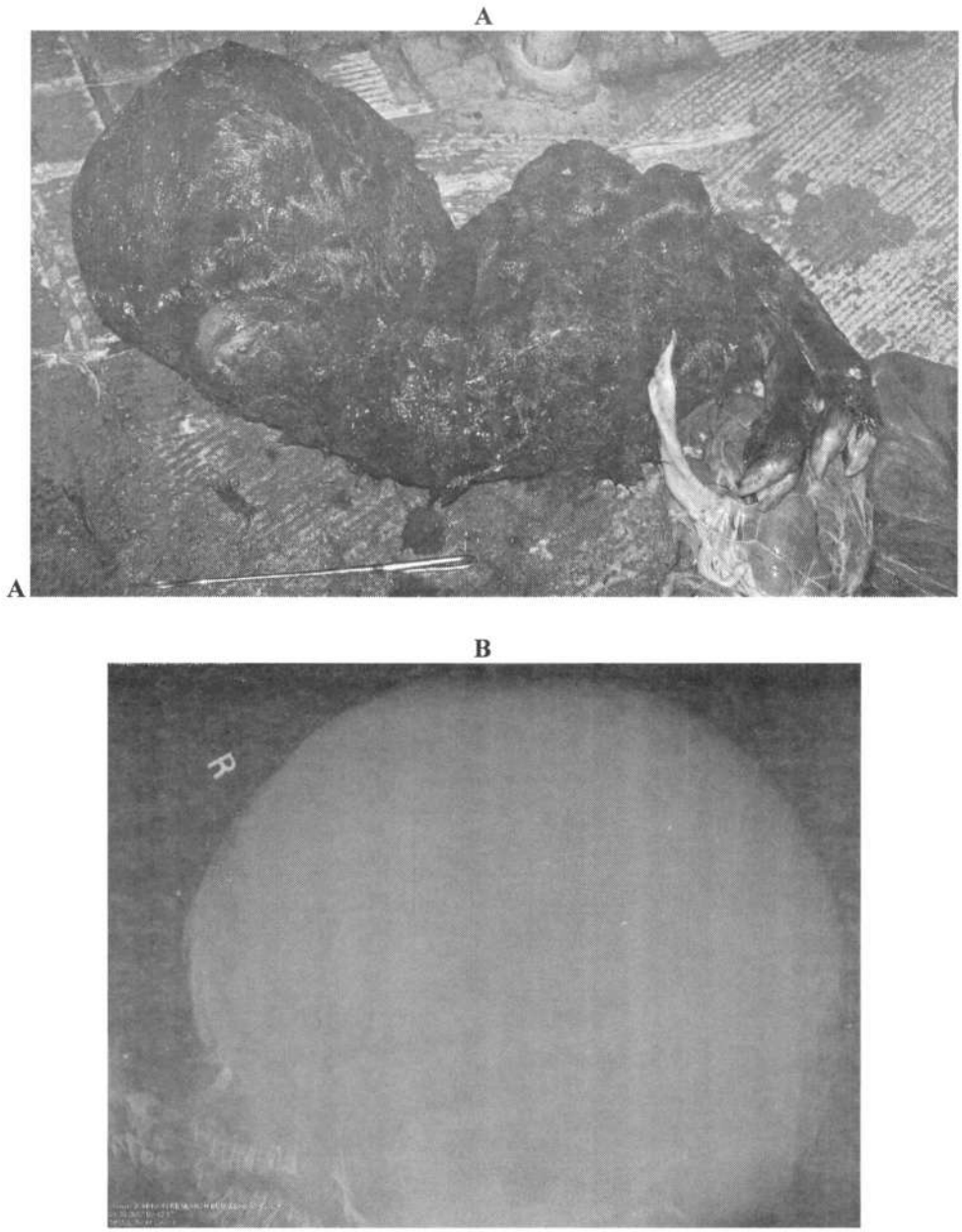

Figure 2. Neuropathic hydrocephalus in a Angus calf that was born dead. Notice the markedly enlarged cranium (A,B) and the loosely organized bones of malformed skull on the radiographic image (B). These images are courtesy of Dr. Brian K. Whitlock of The University of Tennessee.

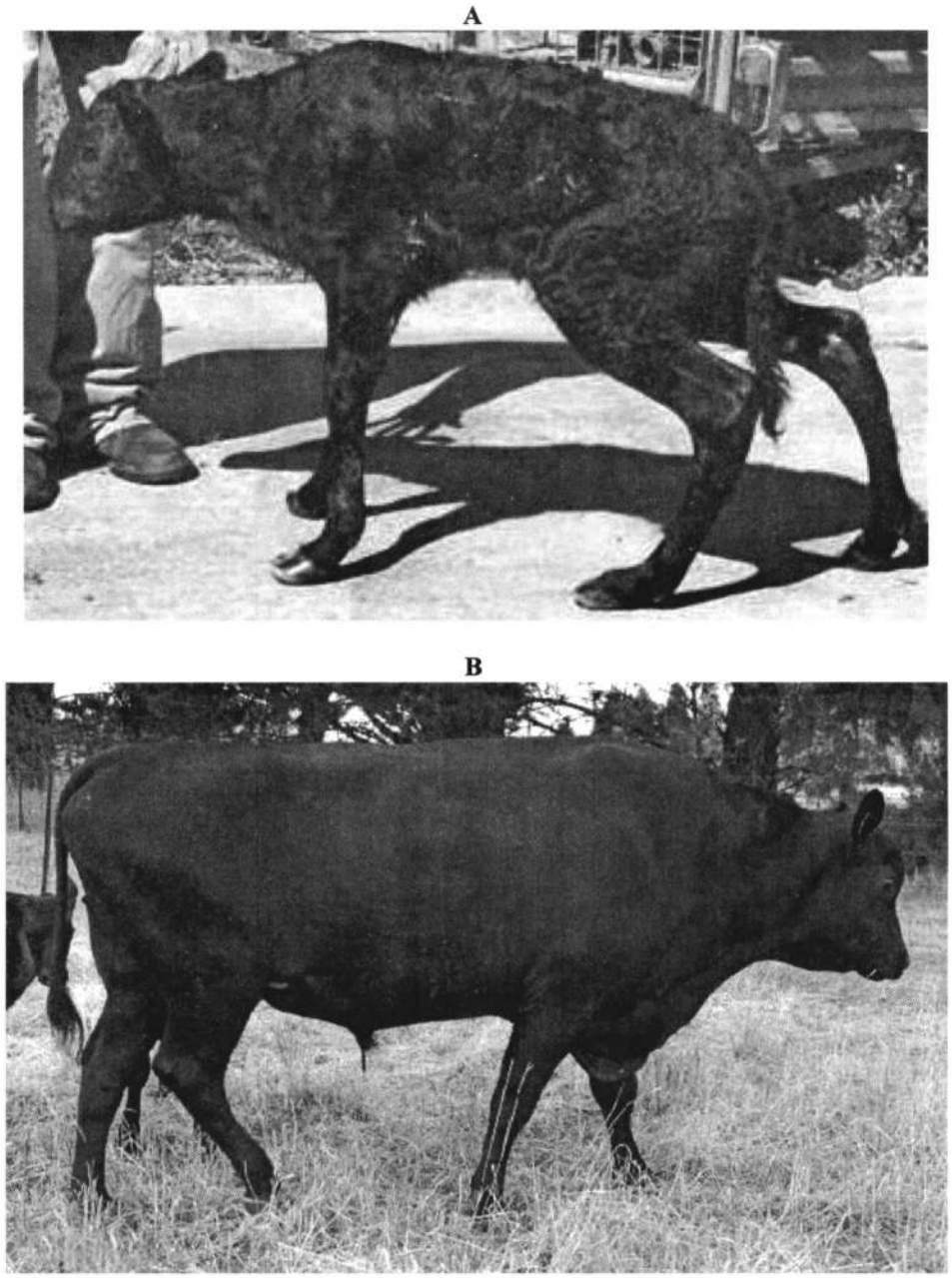

Figure 3. Congenital contractural arachnodactyly (CA) in a day one old Angus calf (A) (Note the angulation of stifle and hock joints due to muscle contracture and hyperextension of loose-jointed fetlocks) and in a six year old affected bull (B).[10]

Complementary care; acupuncture and manual therapy; treatment and diagnosis in production animal medicine and surgery (reproduction emphasis)

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Introduction

Complementary care, specifically acupuncture and manual therapy (chiropractic, osteopathy, physical therapy, myofascial work) are becoming important therapeutic modalities in production animal medicine, especially in the area of pain and reproduction. These modalities are already being used extensively in treating many situations in equine, avian, and small animal medicine and surgery. The acupuncture and manual therapy examination is being used for diagnostic purposes and for therapy. The examination utilizes soft palpation and joint manipulation in attempts to isolate pain and/or lack of normal function that may lead to loss of performance or reproductive status. Acupuncture and/or manual therapy techniques are then applied to the animal in an attempt to regain normal function and reproductive success.

Keywords: Acupuncture, complementary therapy, manual therapy, infertility

Acupuncture and manual therapy are best utilized as complementary care modalities used in conjunction with conventional therapies. Their popularity and use is increasing as a secondary additive-type of therapy especially in those complicated medical/surgical or economically challenging situations. With the rising concerns of drug withdrawal, drug usage, injection site lesions in food animal medicine, and the use of hormonal implants, as well as the known successes, the acceptance of alternative therapies in the eyes of the public as well the rancher and equestrian is increasing.

Some of the areas in which complimentary modalities are being utilized in veterinary medicine include, but are not limited to:

Diagnostic acupuncture

Acupuncture and manual therapy techniques can be utilized in large and small animal medicine for therapeutic and diagnostic purposes. Specific acupuncture points can become sensitive (Ah-Shi points) or painful with certain pathological conditions. Applying pressure to these points and eliciting a painful or sensitive response may help to pinpoint areas of concern or pathology. This is best described and explained by overlapping dermatomes, scleratomes and myofascial trigger points. Shu points or transport points are located along the "bladder channel" and often help to diagnose visceral pathology, via the visceral somatic neurological pathway. They can also be used to treat abdominal and thoracic disease as well as reproductive failure. Some of the most commonly used examples of diagnostic acupuncture include:

- Cardiac disease (high mountain disease, pericarditis, endocarditis, myocardial dysfunction):
  - Sensitive points include: BL-14, 15, ST-10, 11, PC-1, CV-14, and CV-17.
- Lung disease (pneumonia, pulmonary hypertension, any type of lung pathology that may lead to pulmonary pain):
  - Sensitive points include: BL-13, 14, 15, 16, 17, 41, and 42, LU-1
- Gastrointestinal ruminant:
  - Rumen: BL-43-01, ST-36, SP-16 (left side), ST-36
  - Abomasum: BL-18, 19 (right side)
  - Small and large intestinal tract: BL-21, 22, 23, 25, 26, 27, and 28, ST-36
- Gastrointestinal other species:
  - BL-18-26, ST-36
- Liver:
  - BL-18, 19, 20, 43, 44, 45; often these points will be sensitive bilaterally but seem to be most sensitive on right side.
- Ovary:
  - BL-22, 23, GB-26; Often the more intense response (pain) will be on the side of the affected ovary, i.e. cyst, retained corpus luteum; this is most obvious in the mare.

- Uterus:
    - BL-26, 27, 28, GB-27, SP-18, BL-30; diseases of the uterus that can make these points sensitive are pathologies including, endometritis, pyometra, uterine trauma, and uterine torsion.
- Udder:
    - SP-18 (front quarters) located just dorsal to the milk vein. BL-30, (hind quarters) located bilaterally to the sacrum in a bone indentation, lateral and ventral to the last sacral foramen, unreliable.

Reproduction

Acupuncture and manual therapy are being used in conjunction with conventional therapies for those refractive non-breeders including cystic ovaries, early fetal loss, dystocia, retained placenta, anestrous, estrus synchronization, loss of libido, failure to ejaculate, poor semen quality, and failure of semen to freeze and thaw. Acupuncture and manual therapy techniques and methods are numerous and there is a wide range of variability to each. Some of the techniques include: dry needling, dry needles stimulated with moxa (*Artemisia vulgaris*) which is lit and used to heat the area around the needle or the needle itself, electrical stimulation and physical movement of the needle, aquapuncture, laser therapy, motion palpation, chiropractic manipulations and physical therapy.

Treating loss of libido as well as a decline in semen production has proven to be quite successful by utilizing needles, moxa, electrical acupuncture, and aquapuncture. When considering the use of acupuncture or manual therapy in reproductive disorders it is important to focus on the innervation of the organs involved as well as hormonal influences. Often reproductive failure is secondary to an undiagnosed lesion that has nothing to do with the urogenital tract. Painful pathology can result in reproductive failure secondary to loss of normal neurological function to one or more reproductive organs. A good example of this is the stallion that cannot maintain an erection or will no longer mount to breed. When cases with this history are examined via western medicine or complementary, they are often found to have lumbo-sacral pain and dysfunction. Once a lesion is isolated and treated with conventional or complementary therapies reproductive status often returns to normal. There are numerous reported cases that involve males that have the history of reproductive decline as well as a decreased libido that when treated for lumbo-sacral pain and dysfunction returned to normal reproductive behavior and success. Extending the pathology from the lumbo-sacral region, a male or female animal with severe back pain (active NMDA receptors, wind-up) will often have complete reproductive failure. To regain reproductive function the etiology behind the wind-up and pain must first be treated and the pathology eliminated. Trying to breed a mare in wind-up or utilize a male for breeding or collection that is experiencing the same can be unsuccessful for years without proper treatment.

Acupuncture and manual therapy for reproductive issues is a neuroanatomic approach. Specific spinal and sacral segments must be evaluated and treated according to the spinal nerves that exit at that location and the organ they innervate. For instance the lesser splanchnic nerve exits the sympathetic trunk near L-1, 2; pathology in this region may affect kidney and/or adrenal function thereby altering neuro-hormonal reproductive status. These spinal segments must be evaluated and treated if pathology exists. If there are lesions in specific areas coursing along spinal segments and the nerves leaving that segment are affected then normal function of the specific organ innervated by that nerve will be impaired leading to loss of normal function and possible reproductive failure. Often the case may involve numerous areas of pathology; all must be treated prior to the return of normal reproductive status. It is rare that one area of pathology will result in reproductive loss.

Evaluation of hormonal control must be considered as well. The pituitary gland and hypothalamus are under constant control and stimulation from organ function, be it positive or negative feedback. Balanced pituitary and hypothalamus function is essential for the treatment of reproductive disorders. Even though these organs cannot be specifically acupunctured, there are techniques that can be used to help induce neuro-hormonal feedback. Specifically parasympathetic or sympathetic influence can be initiated by acupuncturing points around the pituitary/hypothalamus vascular supply near occiput C-1 level. Acupuncture at these sites may indeed influence the pituitary/hypothalamus function by increasing parasympathetic input to the gland and influencing production of thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) through the adenohypophysis and gonadotropin-releasing hormone (GnRH) through the hypothalamus. The posterior pituitary may also be influenced through regional point selection and help regulate the production of oxytocin and vasopressin (ADH). Points commonly used for pituitary/hypothalamus stimulation are BL10, GB20, TH17, and YinTang. Other points cited include GV1, GV4, CV1-5, these are points located over reproductive organs. Those points stimulating adrenal innervation include BL23, 25, 31-35.

Regardless of the reproductive issue, the approach to all veterinary patients should be the same. The patient should always have a complete in-depth western medical examination and evaluation. After a medical

diagnosis has been made and addressed or routine therapies have failed the animal can then be evaluated for the use of alternative or complementary therapies. The patient should be evaluated first by the diagnostic acupuncture examination (DAPE) or manual therapy examination evaluating the entire body, including gait analysis, motion of individual joints, the sympathetic and parasympathetic nervous system and reproductive organ health. As previously discussed, many reproductive failures may be due to loss of normal neurological input to a specific organ. This situation can be secondary to a specific lesion on the vertebral column or chronic pain secondary to a musculoskeletal disorder. It is for this reason that each animal must be evaluated completely regardless of the primarily reproductive concern.

Examples of this include erectile function and ejaculation. Erectile function is primarily regulated by the parasympathetics with the striated muscle aspect under the control of somatic innervation. Emission of the ejaculate is under the primary control of the sympathetic nervous system. Therefore when evaluating such disorders in the male, acupuncture should be directed at the innervation of the penis as well as that portion of the nervous system with autonomic, sympathetic or parasympathetic control. If dealing with failure to ejaculate it would be appropriate to first evaluate the entire back of the animal regardless of species looking closely at the lumbo-sacral region as well as the sacrum. This region must be evaluated for motion, pain and normal function. Any restriction in this area can result in malfunction or decreased function of the pudendal nerve. The pudendal nerve exits in the dog from all three sacral nerves (S1, 2, 3). In other species specifically other large animal species, not ruling out slight differences, the pudendal nerve exits primarily at S2, 3, 4. Function of the pudendal nerve is essential in reproductive performance in the male and the female.

The pelvis and sacral region of all veterinary patients must be evaluated for function and then stimulated via manual therapy and or acupuncture. The pudendal nerve gives rise to the caudal rectal nerve that innervates the external anal sphincter and levator ani muscles all to be evaluated for such conditions as urine pooling in the female. The perineal nerve also arises from the pudendal and innervates area such as the muscles of the penis and perineal region as well as the vestibule and vulva. Also arising from the pudendal is the dorsal nerve of the penis. The dorsal nerve of the penis courses through the ischial arch to the dorsal aspect of the penis or clitoris and continues to the glans penis and ends at the apex of the glans or clitoris. Arising from the sacral plexus is the caudal gluteal nerve which courses with the pudendal allowing for nerve cross talk in its intra-pelvic pathway as it supplies the perineal branches to the skin around the anus again a concern with urine pooling or prolapse of the rectum and or the vagina. These nerves also can have effect on the penis in such conditions as phimosis or paraphimosis. It can be seen by this example that evaluation of the sacrum and stimulation of the sacral region, one can achieve parasympathetic stimulation from the pelvic splanchnic or sacral nerves from S2, 3 thus stimulating or regulating reproductive function.

Mares often present for the primary concern of loss of performance or speed, or the inability to pick up or hold a lead. When further evaluating the primary complaint, loss of performance, the history of reproductive failure may arise. By evaluating the primary complaint by means of a thorough evaluation the basis behind the reproductive failure may become evident. There are many situations in which there may not be a specific western diagnosis that is leading to the drop in performance and reproductive use. In these situations approaching the case via complimentary or alternative methods using the techniques discussed can often be successful. The nervous system is reliant on exact input from mechanoreceptors (MR) and muscle spindle cells (MSC) for accurate integration into the system to allow for movement and normal reproductive function. It is important to evaluate the peripheral limbs as well as spinal segments in an evaluation. When central nervous system (CNS) input is altered, via pathology affecting MR or MSC such as, restricted motion or pain, the CNS cannot respond normally to the altered input giving rise to an altered response. The altered response may be severe resulting in an altered gait, musculoskeletal function, or loss of reproductive performance. By diagnosing this altered input in the techniques discussed and treating it via western medical approaches as well as complimentary therapeutics it is possible to regain accurate receptor stimulation and neurological afferentation leading to a reproductively and mechanically sound animal.

Completing a thorough examination not only involves the peripheral limbs, sacro-pelvic and lumbar regions of the body but also the thoracic spinal segments. Insuring normal motion and function of the thoracic spinal segments insures normal function of the greater splanchnic nerves arising from the sympathetic trunk at T-6-13 and the lesser splanchnic nerve from L-1-2 which innervate reproductive organs as well as the adrenals. Looking at the equine as a case example, realizing other species can be evaluated in the same protocol with anatomical differences we can approach the nervous system and possible dysafferentation resulting in reproductive failure.

Lumbar vertebrae

As a quick anatomical review of the lumbar vertebral segments, there are in most horses six lumbar vertebrae. The angle of joint facet is vertical and there are six articulations on the cranial lumbar vertebrae and ten on the caudal. Joints that are often overlooked that may have local as well as systemic pain issues are the intertransverse joints, giving rise to the ten articulating joints in the caudal lumbar vertebrae. The intertransverse joints are most commonly located bilaterally and lateral, to L-4, 5, and 6. They are responsible for adding power in the push off as well as stability of the hind quarters. The lumbar region is one of the most concentrated regions of the body for pain and loss of motion. Many of the complaints that the clients may express are: sudden bucking, painful under saddle, loss of push off, loss of ability to turn tightly, hock pain, failure to drop in the rear, overall body pain, lack of performance, lack of desire, and reproductive abnormalities including urine pooling and retention of uterine fluid. This is an area in which the DAPE can be very helpful yet very confusing at the same time. There is often local pain over the lumbars especially over BL-23, between the second and third lumbar vertebrae the area of the exiting of the lesser splanchnic nerve. Other pathologies related to this area (L-1,2,3) through the DAPE are, stifle pain, hock pathology, endocrine disorders, overall back and sacral pain, contralateral forelimb pain, spinal pain, bladder pain, urinary disease, psoas muscle pain, reproductive abnormalities, and local bruising or overall sore back from overuse.

The DAPE serves only as a guide to pain and myofascial dysfunction; most pathology in this area is isolated via motion palpation. Motion and soft tissue palpation in the lumbar spine is critical in evaluation of restricted motion. Often ultrasound is required to better visualize the extent and severity of the pathology present. Motion palpation is best done by evaluating lateral motion bilaterally of the lumbar vertebrae. Grabbing the base of the tail with one hand and placing the other against the facet of each vertebral body, lateral motion can be evaluated thereby detecting loss or restricted motion or pain associated with motion. Lumbar restricted motion can often be improved by motion palpation only. Other pathologies that these lumbar vertebral restrictions may be related to are: L-1, reproduction failure especially ovarian disease or retained testicle; L-2-3, kidney related problems, patella, stifle and sacroiliac pain. Any type of loss or restricted motion in the lumbar vertebrae can result in lower back, sacral, intertransverse joint and L-S pain. The intertransverse joints are often affected by DJD and can be a source of chronic non-responsive pain. These joints can both be evaluated and treated by ventral and cranial motion just lateral to L-4, 5, 6. Pain or resistance with this pressure can be an indication of intertransverse joint pain and loss of function.

Sacroiliac (SI) joint

The SI joint is unique in that it has both a hyaline cartilage aspect on the sacral articulation and a fibrocartilaginous aspect on the ileum. The joint sits at a 65 degree angle in the horse and is supported by many ligaments and supportive tissue. The SI joint is gaining recognition as a joint that can be the source of much inflammation and pain as well as lack of motion resulting in extreme loss of performance. The primary etiology of disease in this area is often related to strain or sprain of the surrounding supportive tissue giving rise to loss of motion unilaterally or bilaterally. It is often recognized by failure of the tuber sacrale (TS) or tuber coxae (TC) to drop or rise. The SI joint can have a dorsal or ventral fixation/restriction (loss of motion) as well which can be unilateral or bilateral. These restrictions can be diagnosed often by watching the horse move or by applying pressure to the tuber coxae and trying to motion the joint in line of articulation. Motion palpation bilaterally is often the best means to regain movement in this joint. It is common that severe SI issues may have to be addressed with western medical approach i.e. injecting the joint, to help relieve inflammation present and then continuing with complementary care to maintain good joint health. Chronic or acute SI pain can cause severe motion restriction and inflammation affecting the sacral nerves which can result in dysafferentation of the sacral nerves and parasympathetic's severely affecting reproductive status.

Sacrum and pelvis

This area is by far one of the most complex and difficult regions of the horse to diagnose and treat. There are many issues and combination of issues that can be taking place. Often pelvic and sacral issues are secondary to overwork, or pain elsewhere in the body. Diagnosis of inflammation and pain over the pelvis and sacrum are best done by motion palpation and the DAPE as well as a routine western medical lameness evaluation and ultrasound. There are three basic categories to pelvic abnormalities or lack of motion. Category I is torsion of the pelvis without osseous misalignment of the SI articulations. This is a twisting and tension pull through the SI joint. Often this is secondary to other areas of pain including shoulder and girth. On the DAPE if there is tenderness and a positive area over the shoulder region at LI-16, GB-21, ST-10, or SI-9, consider a twisted pelvis that may involve not only pelvis but sacrum as well. This can be evaluated by checking DAPE over the pelvic region and by doing motion

palpation and checking for a loss of motion in pelvic rotation. Category II is the basic SI joint loss of motion. This type of pathology most commonly results in loss of balanced gait, painful gait, and lower back pain. Category III has no SI involvement but involves a rotation of the sacral pelvic complex (L-S junction) with L-6. This rotation often results in extreme burning pain in the lower back and the lumbosacral (L-S) joints. Category III pelvic condition should be high on the differentials in those horses that drop in the pelvic region with the DAPE or show extreme tenderness in the LS region. The sacrum is often involved secondary to pelvic categories as described above. A sacral problem is most often seen by the client as a horse that tends to carry its tail to one side consistently.

Clinical applications

Complimentary care is becoming an extension of a western medical approach to the veterinary patient. Complimentary care should not be limited to just one modality but used to provide a complete diagnostic workup and a complete therapeutic approach involving evaluating the patient as a whole body and not as a specific organ failure. Therapy to treat any reproductive loss or problem should be a combination of manual therapy, western medical approaches and the use of acupuncture as well as many other modalities. Below are a few reproductive disorders that were treated in this fashion, utilizing a neuroanatomical approach to acupuncture.

Uterine/vaginal prolapse (primarily large animal). Place needles prior to attempting to reduce the prolapse and run electrical acupuncture at a low frequency setting (GV-2, GV-3). This protocol seems to help with a release of oxytocin as well as endorphins. Other points to utilize BL-23, 25, 28, 29, 30, GV20. Electrical acupuncture from BL-23 to BL-30

Anestrus, lack of ovulation (can be used to help potentate ovulation after AI). Moxa BL-22, 23, 24, 26, laser or needle if possible SP-6 and SP-9, ST-36 and KI-1. After moxa of BL-22 and 23 needle and/or inject vitamin B-12 or saline into the AP points. It should be noted that the author is not utilizing aquapuncture in food animal species, following guidelines set forth by the quality beef assurance program. Dry needle techniques, electrical stimulation, and moxa are the most common modalities used.

Cystic ovaries. Always treat with conventional therapies if possible and add acupuncture and manual therapy techniques as described above as a complementary treatment. If conventional therapies cannot be used then increase the frequency of the acupuncture. BL-23, 24, 25, 26 bilaterally with ST-36, TH-22, and GV-1.

Retained corpus luteum and pyometra. Identify side with the retained corpus luteum and needle BL-22, 23, 24, ST-36, with GV-1. If no response or if used without other therapies then use electrical stimulation.

Mastitis. Mastitis is a disease in which many practitioners report they have never seen a noticeable difference if acupuncture was utilized therefore it is not suggested that acupuncture be used as the treatment of choice for mastitis. For completion of the notes or to stimulate others to better research this field it is being included here. It is best to needle and treat the same side of the cow as the side of the mastitic quarter. If possible, when antimicrobial therapy is used according to regulation and administration directions, inject the desired acupuncture points with the antimicrobial selected. For example, if ceftiofur (not recommended for the use for mastitis) is used as a systemic antimicrobial therapeutic modality, then use it intramuscularly in the acupuncture points BL-30 and BL-54. Then needle the following: front quarter SP-17, 18, 21, and ST-12; rear quarter BL-30, SP-12, BL-49, KI-10 and CV 2, 3.

Bull infertility. This includes loss of libido, loss of ability to mount, and poor semen quality. BL-22, 23, 31, GV-3-01, GV-4, CV-2, 6, GV-1, CV-1 with moxa at BL-18-28, always following a complete musculoskeletal examination.

Example case.

Signalment: Four year old Horned Hereford bull.

History: At the age of 24 months began to show an increase in the number of primary spermatozoa defects, primarily tight curled tails. By the age of 26 months was settling very few cows and semen not suitable for freezing. Bull was also noted to have a drop in libido. Was placed on long term antibiotics and received multiple breeding soundness examinations showing no improvement. For the last two years sired very few calves. Owner elected as last resort to try a round of acupuncture, had heard about it through semen collection units across the USA.

Examination: Temperature, pulse and respiration all within normal limits, body condition score 6 of 9, no gross abnormal physical examination findings.

DAPE examination: Loss of lateral motion from L1-6 associated with pain and segmental dysfunction, loss of ventral motion bilateral SI joints and associated with pain and discomfort.

Treatment: All western medical approaches had been tried; bull was referred by other veterinarians for possible complimentary therapy.

- Complimentary care initiated, acupuncture and manual therapy:
- Manual therapy done involving motion palpation and manipulation followed by high velocity low amplitude SI adjustments and the use of vibration massage.
- Acupuncture performed
    - Points used in this case:
    - GV-24—Calms and quiets parasympathetic input, has some thought of influencing the pituitary for balancing affects.
    - Da Feng Meng—Centers, parasympathetic stimulation
    - BL-18-26, Somato-visceral arch, stimulation of kidney, spleen, adrenals, reproductive organs
    - GV-1—stimulation of perineal nerve as well as pudendal influencing organs of reproduction.
    - CV-1— stimulation of perineal nerve as well as pudendal influencing organs of reproduction. Has specific influence on the dorsal nerve of the penis as it courses along the ischial arch.
    - GV-4—(Ming Men)—Major point for stimulation of kidney and adrenal innervation.

Summary

In summary, it is obvious to see that complimentary care and therapy is gaining acceptance and strength in the veterinary profession. There is a great need for scientific studies to support the neurology behind complimentary care and to support therapies concerning different pathological processes. Complimentary care is a very complex interaction of the nervous system and involves more than just placing needles into an animal with hopes of generating a response. Each animal must be looked at as an individual and examined accordingly. Cookbook protocols can be utilized but still require an in-depth physical examination and treatment of all issues present. Often the animal's primary complaint has multiple etiologies. For the best success all issues must be addressed both with western medical modalities as well as manual therapy and acupuncture techniques. It is very important to address the issues with direction of neuromodulation and stimulation of the central nervous system.

Suggested reading

1. Clayton HM, Flood PF, Rosenstein DS: Clinical anatomy of the horse. St. Louis: Mosby; 2005.
2. Denois J-M, Pailloux J-P: Physical therapy and massage for the horse. 2nd ed. North Pomfret (VT): Trafalgar Square Books; 2001.
3. Giniaux D: What horses have told me: an essay on equine osteopathy. Cleveland Heights (OH): Xenopnon Press; 1996.
4. Dunn PA, Rogers D, Halford K: Transcutaneous electrical nerve stimulation at acupuncture points in the induction of uterine contractions. Obstet Gynecol 1989;73:286-290.
5. Filshie J, White A: Medical acupuncture: a western scientific approach. London: Churchill Livingstone; 1998.
6. Fleming P: Equine atlas of acupuncture loci. Dade City (FL): Florida Equine Acupuncture Center; 2000.
7. Gatterman M: Foundations of chiropractic: subluxation. St. Louis: Mosby; 1995.
8. Getty R: Sisson and Grossman's anatomy of domestic animals. 5th ed. Philadelphia: Saunders; 1975.
9. Haussler K, editor: Back problems. Vet Clin North Am Equine Pract 1999. Vol 15, No 1.
10. Kendall DE: Dao of Chinese medicine. New York: Oxford University Press USA; 2002.
11. LeBlanc MM: Reproduction-clinical cases. Proc Am Assoc Equine Pract 2006. p. 585-590.
12. Lin JH, Wu LS, Wu L, et al: Aquapuncture therapy of repeat breeding in dairy cattle. Am J Chin Med 2002;30:397-404.
13. Liu IKM, Troddeson MHT: The diagnosis and treatment of endometritis in the mare: yesterday and today. Theriogenology 2008;70:415-420.
14. Maciocia G: Foundations of Chinese medicine: a comprehensive text for acupuncturists and herbalists. 2nd ed. London: Churchill Livingstone; 2005.
15. Schoen AM: Veterinary acupuncture: ancient art to modern medicine. St. Louis: Mosby; 2001.
16. Shmalberg J, Xie H: The clinical application of equine acupuncture. J Equine Vet Sci 2009;29:753-760.
17. Schwartz C: Four paws, five directions: a guide to Chinese medicine for cats and dogs. Berkeley (CA): Celestial Arts Publishing; 1996.
18. Suhling KL, Gentry PC, Fredrick PF, et al: Case study: efficacy of acupuncture sites for delivery of agents to control estrus and ovulation in the mare. Prof Anim Sci 2003;19:317-320.
19. Stener-Victorin E, Wu X: Effects and mechanism of acupuncture in the reproductive system. Auton Neurosci 2010; Mar 27 [Epub ahead of print].
20. Wang S, Zhu, B, Ren X, et al: Experimental study on acupuncture activating the gonadotropin-releasing hormone neurons in hypothalamus. J Tradit Chin Med 2010;30:30-39.

David Letterman's top ten reasons for dairy cow infertility

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Introduction

Numerous factors contribute to the failure of cows to become pregnant. This paper, which is based on scientific studies and observations from more than 40 years of conducting pregnancy examinations for herd managers, categorizes common factors that lead to infertility in cows. Following is a ranking of these factors in ascending order—a "Top 10" listing, à la David Letterman, but with elucidation backed by scientific references.

Keywords: Infertility, cattle, pregnancy rate

Reasons for infertility

10. Early embryonic death (EED). One paper[1] reports that 88% of cows contain a fertilized ovum five days after breeding at estrus. However, only approximately 30% of the cows will be diagnosed pregnant at 42-45 days, when the embryo is implanted in the uterus. Many experts calculate the conception rate as the number of cows pregnant divided by the total number of cows serviced. As reproductive science has evolved, newer strategies suggest that a host of other reasons, besides failure to conceive, contribute to infertility. Some of these reasons[2] include:

- Endometritis, which creates a hostile uterine environment. This issue will be addressed later in this paper.
- Endometrial fibrosis, which creates an environment that will not nourish the embryo prior to implantation.
- Prostaglandin injections that are inadvertently administered to a previously-bred cow.
- Genetic incompatibility between gametes, causing a failure of the embryo to develop.[1]
- Nutritional deficiencies, such as vitamin E/selenium or copper, which occasionally result in herd-wide EED.
- Nutritional excesses, such as high dietary degradable intake protein (DIP), can also be embryocidal.[3,4,5]
- Toxicity from several mycotoxins, including zeralanone, dioxynivenol (DON), ergotamine and aflatoxins, frequently cause EED, in combination with other factors.
- Environmental stress of high temperatures may cause significant EED.

Minimizing the above issues increases fertility.

9. Body condition scores of 2.0 or less on a 1.0-5.0 scale significantly delays the return to normal ovarian cyclicity and causes severe infertility.[6] Management procedures that minimize weight loss at calving contribute to improved pregnancy rates.

8. Inappropriate close-up dry cow rations prior to calving often create severe metabolic diseases at calving.[7]

- A dietary cation-anion difference (DCAD) in excess of +10 causes close-up dry cows to develop:
  - Subclinical or clinical hypocalcemia at calving
  - Retained fetal membranes
  - Delayed involution
  - Endometritis
  - Suppressed immune response
  - Delayed returns to ovarian cyclicity
  - Lower conception rate
- Dietary energy above 1.3 MegCal/kg. fuels appetite suppression. In this condition cows are prone to:
  - Highly mobilized non-esterfied fatty acids (NEFA)
  - Rapid weight loss, which delays return to ovarian cyclicity
  - Displaced abomasum, which is another contributor to excessive weight loss

No dietary factor has a greater positive impact on dairy cow fertility than implementation of an appropriate transitional dry cow program that utilizes a palatable, well-formulated low-DCAD, low-energy/high-fiber diet.

7. Mastitis. It has been reported that cows that have at least one high somatic cell count (SCC) score prior to conception will require 48.7% more time and 0.49 more services to achieve a pregnancy.[8] The odds of a

pregnancy decreased by 44% with a high SCC linear score prior to breeding. Cows with a high SCC during the first 90 days of gestation had a 1.22 increased risk of abortion over cows with a low linear SCC. Mastitis prevention plays a significantly positive role in improving pregnancy rates.

6. Low heat detection rate. Ferguson, et. al. reported that most herds had a heat detection rate of less than 50%.[9] Several reports demonstrate that low heat detection rates can be alleviated by timed AI programs.

5. Endometritis. Cows with endometritis not only have a hostile uterine environment but a series of biological events that affect every aspect of cow fertility.[3,4,10-14]. The primary organisms causing endometritis in dairy cows are *E. coli, Arcanobacter pyogenes, Prevotella sp., Fusobacteriaum necrophorum and Fusobacterium nucleatum.*[10]

E. coli occurs by day 7 after parturition, producing lipopolysaccharides (LPS) and causing serious consequences. The LPS converts prostaglandin (PG) $F_2\alpha$ to PGE by a phospholipase A2-mediated mechanism. Lipopolysaccharides stimulate the cow's immune response, because toll-like receptors attach to the LPS secreted by the *E. coli.* Toll-like receptors flood the uterine environment with cytokines, chemokines, opsonins, antimicrobial peptides and anti-inflammatory proteins such as *a*-glycoprotein and haptoglobin.[11,12]

Oxytocin normally stimulates the endometrial cells for PGF synthesis. Lipopolysaccharides convert $PGF_2\alpha$ to PGE, negating the effects of oxytocin on endometrial cells. Consequently, the increased concentrations of PGE exert a luteotropic effect on the ovary with delayed luteolysis of the corpus luteum, prolonged periods of progesterone influence and a reduced defense mechanism in the uterus. There is also a negative effect on the ovary, the hypothalamic-pituitary axis and the cow's overall general health.

Lipopolysaccharides negatively affect the aromatase cascade, which is a key link in expressing estradiol from androgens. Not only does LPS have a luteotropic effect through conversion of $PGF_2\alpha$ to PGE; lower production of estradiol causes dominant follicles to be smaller and less likely to ovulate because of the interruption of ovary and pituitary interactions.[10] Cows with reduced peripheral estradiol levels also tend to exhibit fewer signs of estrus.

The most severe bacterial effects on the uterus are caused by *Arcanobacter pyogenes.*[10] It expresses a virulence gene, which produces a cytotoxin called pyolysin. Pyolysin kills endometrial, endothelial and stromal cells, thus enhancing uterine disease. If a cow with uterine disease ovulates, its level of plasma progesterone is lower than that of a normal healthy cow's.

Bovine herpes virus IV is also associated with uterine disease. It appears that latent infections may be a prominent feature of this virus. No one will likely determine the prevalence of herpes IV in dairy cows until a vaccine is developed to prevent it[11,15-17].

For cows with endometritis, conception rates can be improved by using a protective sheath on the Cassou gun at a second AI service.[18] However, a protective sheath makes little difference in conception at first service.

4. Sub-acute rumen acidosis (SARA). The mechanism is not well understood, but herds with SARA have lower pregnancy rates. One may deduce the indirect effect that SARA can have on reproductive efficiency by way of lameness and abscesses. However, many veterinarians, including myself, believe that SARA has a direct negative effect on reproduction. Research to address this is needed; there is little definitive information in the scientific literature. Rumen acidosis is diagnosed by using the Penn State Particle Separator Box, ruminocentesis, observations of clinical signs, and butterfat tests. It is often noted that incidence of lameness is more common with displaced abomasums. Addressing dietary issues related to rumen acidosis often improves pregnancy rates and many other production-related problems.

3. Reproduction management implementation. Timed artificial insemination, known as Ovsynch, is the most effective method for reproductive improvement through the synchrony of placing semen in the uterus just prior to ovulation.[15] This has resolved much of the issue of low heat detection efficiency.

The following real-life narrative describes quite well the importance of Ovsynch implementation:

The manager of a 1,000-cow herd had been highly effective in maintaining a high pregnancy rate. However, from one weekly visit for pregnancy examinations to the next, the number of cows diagnosed pregnant decreased by two-thirds. Little in the way of explanation was discovered through an extensive diagnostic investigation that included tests for BVD, leptospirosis serology, urine examinations, review of ration formulations, bulk tank milk urea nitrogen tests and semen integrity. Coincidentally, the employee who had been managing the herd's timed insemination program had recently left for another place of employment. The herd manager took on the responsibilities of administering the GnRH and $PGF_2\alpha$ injections. He immediately discovered that the automatic

syringe used to give the $PGF_2\alpha$ injections was cracked, causing most of the $PGF_2\alpha$ to spray out the side of the syringe barrel onto the ground. Once the automatic syringe was repaired, the pregnancy rate returned to the expected level.

2. Persistent infections from bovine virus diarrhea (BVD). Much has been written about persistently infected BVD (PI-BVD) animals in scientific literature[19] and the lay press, but there has not been adequate dissemination of information about cows developing ovaritis and chronic oophoritis from BVD virus. This is frequently overlooked. Yet, in my experience, it is a big player in infertility in herds with active BVD or a PI-BVD animal. There is no treatment for ovaritis other than a) implementing rigid biosecurity and b) giving the client an informed prognosis that it may be six months after BVD has been eradicated from a herd before optimum fertility returns.

1. Probability. By virtue of probability, about half of all coins flipped will land heads, the rest tails. Continued flips of coins that initially came up tails will again result in about half landing heads up. Probability works much the same with cow pregnancies.

Pregnancy rates are a far better measure of cow fertility than conception rate, bull stud non-return rates or percent cows open. While more sophisticated formulas are used to calculate an overall herd pregnancy rate via various software programs, in its simplest form, the pregnancy rate can be calculated as the Heat Detection Rate X Conception Rate.[10]

Conclusion

Even though the preceding factors are ranked in reverse order of importance, any of them, alone or in combination, can cause significant harm to the reproductive program of a dairy herd.

References

1. Thatcher WW: Characterization of early embryonic death and prevention of pregnancy wastage. Proc Annu Conf Am Assoc Bovine Pract 2003. p. 100-108.
2. Betts DH, King WA: Genetic regulation of embryo death and senescence. Theriogenology 2001;55:171-191.
3. Bondurant RH: Controlling what we can control: limiting embryonic/fetal losses. Proc Annu Conf Am Assoc Bovine Pract 2004. p. 2004-2023.
4. Rajala-Schultz PJ, Saville WJA, Frazer GS, et al: Association between milk urea nitrogen and fertility in Ohio dairy cows. J Dairy Sci 2001;84:482.
5. Melendez P, Donovan A, Hernandez J et al: Milk, plasma and blood urea nitrogen concentrations, dietary protein, and fertility in dairy cattle. J Am Vet Med Assoc 2003;223:628-634.
6. Opsomer G, Grohn YT, Hertl J, et.al: Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology 2000;53:841-857.
7. Goff J: Transition cow immune function and interactions with metabolic diseases. Proc Tri-State Nutrition Conf 2008. p. 45-57
8. Pinedo PJ, Melendez P, Villagomez-Cortes JA, et al: Effects of high somatic cell counts on reproductive performance of Chilean dairy cattle. J Dairy Sci 2009;92:1575-1580.
9. Ferguson JD, Galligan DT: Assessment of reproductive efficiency in dairy herds. Compend Cont Educ Pract Vet 2000;22:S150-S159.
10. Risco CA, Drost M, Thatcher WW, et al: Effects of calving-related disorders on prostaglandin, calcium, ovarian activity and uterine involution in postpartum dairy cows. Theriogenology 1994;42:183-203.
11. Sheldon IM, Cronin J, Goetze L, et al: Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod 2009;81:1025-1032.
12. Herath S, Lilly ST, Fischer DP, et al: Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2α to prostaglandin E2 in bovine endometrium. Endocrinology 2009;150:1912-1920.
13. Williams EJ, Fischer DP, Noakes DE, et al: The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 2007;68:549-559.
14. Benzaquen ME, Risco CA, Archbald LF, et al: Rectal temperature, calving-related factors, and the incidence of puerperal metritis in postpartum dairy cows. J Dairy Sci 2007;90:2804-2814.
15. Peters MW, Pursley JR: Fertility of lactating dairy cows treated with Ovsynch after presynchronization injections of PGF2α and GnRH. J Dairy Sci 2002;85:2403-2406.
16. Meyer G, Bare O, Thiry, E: Identification and characterization of bovine herpesvirus type 5 glycoprotein H gene and gene products. J Gen Virol;1999:2849-59.
17. Ackerman M: Pathogenesis of gammaherpesvirus infections. Vet Microbiol 2006;113:211-222.
18. Bas S: Effect of using protective AI cover sheaths on fertility of lactating dairy cows. Reprod Fertil Dev 2010;22:163-163.
19. Grooms DL: Detection of bovine viral diarrhea virus in the ovaries of cattle acutely infected with bovine viral diarrhea virus. J Vet Diagn Invest 1998;10:125-129.

Time management in a broodmare band

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Abstract

Successful management of a band of broodmares whether it is two or two hundred involves more than just getting the mares in foal. The conservation of time that is available to breed the mares is a crucial factor in any program and its judicial management is important to the long term success of any program with a goal to produce a foal every year from each mare. It is important to remember that the mare is not only being bred for this season but also for the following season and many seasons to come. The time of the year she becomes pregnant this season will have an effect on how much time she will have available to be breed next year. As most of the author's experiences are in the natural covering world of the Thoroughbred that is where the emphasis of this discussion will be. However, it can certainly be adapted to any breeding system that is being used; all of the criteria will still apply.

Keywords: Broodmare, breeding, management

Time management also becomes a useful tool to help the veterinarian and the farm manager to more efficiently use their time, prevent unnecessary examinations, and reduce some of the expenses of the breeding season. The judicial management of time will positively affect all aspects of broodmare management on a farm. It will keep mares producing more regularly, save the farm management time by keeping the management of mares more tightly grouped so that help can be used more efficiently and save the veterinarian time as a larger percentage of the mares will be examined on a single visit.

In order to accomplish the goals established for optimal time management a strategy must be developed by all of the involved parties. The veterinarian, owner, manager and farm help need to understand the goals that need to be achieved and the methods that are being used to achieve them. It is of the upmost importance that the goals are well understood by all parties so that everyone is onboard. If one part of the team is being dragged along, the success of the project will be more difficult to achieve. The first step in this endeavor is to set obtainable and realistic goals. These goals will be determined by the desired end result and the product being produced. Goals will certainly be different for a farm that is selling Thoroughbred yearlings when compared to a farm that is raising Warm Bloods for use as performance horse at five to six years of age. Other farms, for management reasons, may not want foals until March. Any of these management reasons, if planned for, can and should be incorporated in the plan and still allow for better mare management through planning. Once the plan has been worked out and put into place than the implementation becomes the important thing.

The decisions that need to be made before the season starts are; on what date will the first mare be bred, to whom are the mares going to be bred and how are each class of mares going to be managed to obtain optimal results. For the Thoroughbred in North America the traditional start of the season is February fifteenth but some farms may want to wait and start at a later date. As the heart of this system is estrus synchronization, stallion selection should be looked at and mares that are bred to a specific stallion should be spread out a little for easier management. In order for synchronization to work effectively the mares must be cycling, a system of artificial light management must be decided on and started at the appropriate time, so that mares will be ready to cycle at the appropriate time. Once the management systems have been decided on, they need to be put into practice and not deviated from.

The goals of the project are to have a system that will develop a band of mares that produce a foal every year at the most appropriate time to be an economical commodity. Broodmares, even when managed aggressively, will tend to have a drift in the foaling dates which causes a loss of time and a reduction in opportunities for a mare to become pregnant. In a large study of breeding records it was found that the average drift in foaling dates in mares bred in subsequent seasons on well managed farms was 13.4 (±23.2) days.[1] This drift can be minimized if mares are bred and become pregnant within 25 days of foaling.[1] The control of the drift is important as mares will work their way off the calendar and then must be passed because of foaling dates that are too late.

The achievement of a successful time management program must start well before the start of the breeding season as it is important to have mares ready to be bred when the season begins. Mares should be checked in the fall for pregnancy. All mares should be examined, even the ones that were thought to be barren; every year there are mares found to be pregnant that were reported as barren at the end of the season. The barren mares should be examined and cultured while they are still cycling in the fall. At this time their Caslick suture can be evaluated and repaired if need be, and other problems like possible urine pooling, severe endometrial cysts, torn cervixes and positive uterine infections can be evaluated and treated before the upcoming season. The mare's hair coat and body condition should be evaluated and her general health evaluated. This can be done quickly with just general

observation, as most mares on a well managed farm will be in good condition, however, sometimes a different set of eyes will see things that are missed by someone who looks at the mare every day. Over- or underweight mares should have their feeding altered to address their individual condition. It is important to have a mare enter the breeding season in the proper body condition and addressing those problems is much easier done before the season starts than trying to correct a problem during the breeding season. Hair coat is frequently a good indicator of general health. An unhealthy or shaggy hair coat can be a sign of conditions such as Cushing's syndrome and pituitary tumors.

After evaluation, the mares can be divided into groups that, when possible, will be maintained throughout the breeding season. In large farms it is wise to have barren, maiden, and foaling mares separated. In farms with a large number of foaling mares, they should be divided by foaling dates so that they will remain in the same groups throughout the season. Keeping mares in the same social groups will reduce the stress level as they can get the dominance order worked out before the breeding season begins.

In late November or early December the mares that are going under light should be quartered in facilities with proper lighting conditions.[2] There are several systems that work well for artificial lighting the choice will depend on the management style of the farm. Mares can be put in stalls, sheds or paddock confinement with artificial light. The mare should receive a total of 16 hours of light with artificial light added after sunset. It is important that there be a period of darkness, the lights must be on a timer and not left on all night. Traditionally mares have been exposed to at least sixty days of lights from the time they are started until first breeding. In the past several years there have been several alternative methods and treatments for stimulating a mare to pass through the transitional estrus period. These protocols have been well-described and will not be elucidated here. The object of whatever system is used is to have a mare cycling, so that she can be synchronized for breeding at the start of the breeding season. Early foaling mares can also be positively affected by exposure to artificial light. If mares that foal before the fifteenth of April are exposed to artificial light on the same schedule as the open mares it will positively affect their reproductive performance. The exposure to light has been shown to encourage the mares to foal about ten days early and more importantly it reduces the incidence of post-foaling anestrus.

For the purpose of this discussion the start of the breeding season will be February fifteenth. A perfect start to the season would be for all mares that are open, foaling, barren and maiden to be bred on this date. Of course, that is not possible but to think of this as a goal is a good way to set the tone for the season. If mares are put under light on December first, the teasing should begin by mid-January, with the maiden and barren mares. The mares should be examined for follicular activity when they show estrus or at least before they are started on any estrus synchronization program. Mares with no follicular activity should be evaluated before they are entered in the program. Many farm managers will elect to start the mares anyway and often this is the proper decision as these mares will have another twenty days under lights before they will need to ovulate and frequently they will respond well. If they don't respond, the evaluation will allow the veterinarian to make a proper diagnosis and implement an appropriate treatment without delay.

In the author's opinion the gold standard for estrus synchronization is the use of progesterone and estradiol in oil as a daily injection. The use of this product has stood the test of time. It has been used for over thirty years and on thousands of mares. It is inexpensive, a little more trouble than other systems but in the experience of the author, the side effects are minimal, limited to sore necks and some mares being difficult to catch, and the results are reliable. The protocol is simple and straightforward. The object of the treatment is to completely shut the mare's ovaries down so that she is starting with no significant follicular activity and when treatment is stopped she will develop a primary follicle at a normal rate. This process from the start of treatment to ovulation will take between nineteen to twenty-one days with very few mares ovulating outside that period.

The protocol for this procedure is as follows: The mare is palpated on day one to ascertain the degree of follicular activity. She is then given an injection of three ml of progesterone and estradiol that contains 150 mg of progesterone and 10 mg of estradiol in oil.[3] This treatment is continued for ten days and on the tenth day the mare is also given an injection of prostaglandin and palpated. The prostaglandin is given to destroy any luteal tissue that is remaining from an ovulation that may have occurred during the treatment. The reason for the palpation is to determine if there is any follicular structure on the ovary. The presence of a follicle is important to be aware of because its presence will cause the mare to show signs of estrus earlier than mares that do not have a residual follicle. However, the mare will make a second follicle while the original one regresses and this can be very confusing to someone who has not worked with this product in the past. Most mares will come into heat about day seventeen and be ready to breed about day twenty. Ovulating drugs may be given if the mare is to be covered earlier and they have the same effect as they would in a mare that is not being synchronized. If a group of mares are being synchronized it is best to spread them out so that they are not all being bred on the same days. It is common for larger farms to group mares and then leave two or three days between the start of synchronization so that there are

no more than five or six mares in a group and these mares are going to different stallions, with no more than two mares per group going to the same stallion. This grouping may not be as important when artificial insemination is being used, if semen is available to a large number of mares on any given day.

The first group of barren and/or maiden mares should be started nineteen days or so before the optimal breeding date and then any additional groups can be started at two or three day intervals. This will allow the farm to have an opportunity to breed all of the maiden and barren mares in the first two weeks or so of the breeding season. Mares that have foaled before the start of the breeding season may also be synchronized with the same schedule as the maiden and barren mares.[4] The author has done this with excellent results when using mares that have been through their foal heat prior to the time that the maiden and barren mares are ready to be synchronized. It is the author's opinion that if the foaling mares have not had a foal heat, it is better to let them have that heat and then handle them in a different manner. Foaling mares must be handled aggressively, remembering that this year's foaling mares will make up the majority of next year's barren mares in most herds.

Foaling mares that are not synchronized and that have their foal heat after the start of the covering season should be routinely examined for the first time at about seven days after foaling if there is no medical or other reason to examine them before that time. If mares are examined earlier than seven days the mare will not have time to recover from the trauma of foaling and it is difficult to evaluate the severity of any damage that was done during foaling. Mares have an amazing ability to repair after foaling and should be given a chance to do so before any intervention is attempted. Caslick procedures may be replaced before seven days if the mare has sufficiently healed that the Caslick suture line will heal. If the vulvar lips are infected or badly traumatized it is better to wait to replace the Caslick as the suture line will not heal.

At seven days the foaling mares should be examined be with a speculum. This will give the veterinarian an opportunity to visualize the vulvar lips, the vestibule and vagina for lacerations. Cervical lacerations, inflammation, discharge and urine pooling can also be observed. If the examination warrants it an endometrial culture and cytology may also be performed. The uterus and ovaries should then be palpated. The ovaries are evaluated for size, consistency and follicular activity. Granulosa cell tumors can often be formed during pregnancy and are found at this time. The uterus should be evaluated for size, consistency and tone. Post-foaling uterine hemorrhage that was not severe enough for the mare to show clinical signs is often found at this time. After through palpation, the mare's reproductive tract should be examined by ultrasound and particular attention should be paid to the mare's uterus. The presence of uterine cysts can be evaluated and recorded along with the presence of fluid in the uterus. The ovaries are evaluated and any abnormalities found during palpation can be examined further. At this time a judgment can usually be made as to whether or not the mare should be considered for foal heat breeding. If the mare is to be considered for breeding, further examinations should be performed very critically. It is the author's opinion that in order for foal heat breeding to be successful the mare must meet certain criteria. First, she should not be infected or look very inflamed, she should have minimal fluid in her uterus, and lastly should not be bred until she is at least ten days from foaling. If these criteria are adhered to, success at foal heat will be very close to the conception rate at second heat or later heats. If a mare is not bred on foal heat, then it needs to be decided on whether to short cycle her from her foal heat ovulation or to let her return to estrus on her own. Whichever route is decided on, it is important to know when the mare ovulated from her foal heat so that her return to estrus can be anticipated. Foaling mares are frequently difficult to tease, so it is important to keep track of when they are due back in heat. The reason it is important to wait until ten days post-foaling to breed a mare is because the conception rate is so low before ten days that the overall time will be saved for a herd of mares by short cycling these individuals. Mares with uterine infections, severe trauma or other more severe problems should be treated and not rushed into breeding when there is not a good chance for conception. Once a mare is bred the breeding should be respected even if the chance of conception is low, so again time is saved by waiting and breed when the mare is as near "right" as possible.

From the beginning of the breeding season onward the most important procedure on the farm is teasing. In recent years as more modern technologies have been brought into play, teasing has been deemphasized. In the author's opinion this is a mistake. The author is very lucky in that he has been practicing in an arena where the mare must be covered by the stallion and this has kept teasing alive. A competent teasing person is a great asset to the veterinarian. With the use of a teaser the veterinarian will know how long the mare has been in heat, how well she is showing estrus and whether she is on the way in or out of heat. Follicle size and uterine edema are important but paying attention to the teaser adds valuable information and in many instances will keep everyone out of trouble. It has been suggested by some workers that teasing also improves mare's uterine health by causing oxytocin release which helps to clear any fluid in the mare's reproductive tract.

After the mare has been bred and ovulation has been confirmed, regular examinations should be scheduled so that the mare's performance can be frequently evaluated and if things are not progressing normally the

veterinarian can intervene in an attempt to get things back on track. The author likes to examine mares the first time after breeding on the fourteenth day from OVULATION not breeding. This is important as mares have certainly become pregnant that ovulated as long as seven days post-breeding and if these mares were examined on day fourteen from breeding many pregnancies could be missed. If a mare has more than one follicle that ovulates asynchronously it is important to examine them from the day of the last ovulation. The author picks day fourteen because the embryos are not fixed at this stage and if multiple embryos are diagnosed you will have time to deal with them before fixation. If there is any chance of multiple embryos the author will usually examine the mare again at eighteen days to be sure that nothing was missed. If all appears to be normal the next examination should be around twenty-eight days, this will allow the veterinarian to see a heartbeat in the embryo and if the embryo appears abnormal there will still be time to intervene before the formation of endometrial cups. The next examination is usually performed about forty-two days as this is the date when pregnancy is considered diagnostic by insurance companies, sales companies and many breed registries. The mare is examined again at between sixty and seventy days for fetal sexing or just a final pregnancy determination. Later pregnancy examinations are performed when needed for sales, insurance examinations, payment of stud fees or any other management reason when an absolute diagnosis of pregnancy is needed.

We have discussed the methodology for achieving high reproductive performance but how should the outcome be judged? Classically and ultimately the final judgment is the live foal rate. This is in fact a very important measurement but in order for a high live foal rate to be sustained year after year other factors must be taken into consideration. The first measurement that is a significant measure of performance is the conception rate. An even closer look that is frequently used as a measure of performance is the per cycle pregnancy rate and then it can be broken down even farther into breedings per conception. These are the measurements that are all frequently referred to when breeding efficiency is being judged and they are all important. A less frequently used measure of breeding efficiency and, in the author's view, an important measurement in sustaining all of the other parameters is a measure of days open. Days open refers to the days that a mare is not bred compared to the days that she is eligible to be bred. For example if the first day of the breeding season is February fifteenth, then every day after the fifteenth of February would be an additional day open when measuring a maiden or barren mare that has been on the farm under lights. Foaling mares are measured from their foaling date and every day after that is considered to be one day open. This figure is important because as mentioned earlier if the days open interval exceeds twenty five the mare foaling date will start to get later and the mare will eventually work their way off the end of the calendar. The number of years that elapse from the start of this movement is determined by how rapidly it occurs and how early the mare begins her broodmare career. Mares that begin foaling in January will certainly have more time to recover from problems of the foaling season before they have to skip a year because of their foaling dates. High performing farms realize this and work hard to keep mares foaling early. The author has one farm that has a goal of not only a ninety percent plus conception rate but also of having fifty percent of its foals on the ground before the fifteenth of February. The farm certainly doesn't reach that goal every year but at least it has a goal that can be worked toward. Many broodmare operations may not want foals that early in the year and that is fine but goals should be set so that there is a plan to follow.

In the author's opinion the broodmare business is production medicine very similar to any other livestock production business. One major difference that hurts the production numbers in the horse industry is the difficulty of culling. For the best production culling needs to be done. This is well-demonstrated when the reproduction records are examined on operations that are producing horses of lower value on a commercial basis.[5] Culling of purebred horses often must take other factors into consideration besides just reproductive performance. Even on a commercial farm when sentiment is not a factor, mares with bad production records may be kept because the high value of their offspring will offset the expense of the years of no production. In these instances the management and veterinarian must just do the best they can.

The added expense, if any, of intensively managing a broodmare band is usually more than offset by the increase in value of an older individual to sell or train and better performance from the broodmare band. An added benefit of more intense management is usually more efficient use of the veterinarian's and farm's time, and a more condensed breeding season.

References

1. Bosh KA: The influence of management and veterinary practice on reproductive efficiency in the Thoroughbred mare [dissertation]. Lexington: University of Kentucky; 2006.
2. Loy RG: Effects of artificial lighting regimes on reproduction patterns in mares. Proc Am Assoc Equine Practit 1968. p. 159-169.
3. Loy RG, Pemstein R, O'Canna D, et al: Control of ovulation in cycling mares with ovarian steroids and prostaglandin. Theriogenology 1981;15:191-200.

4. Loy RG, Evans MJ, Pemstein R, et al: Effects of injected ovarian steroids on the reproductive patterns and performance in post-partum mares. J Reprod Fertil Suppl 1982;32:199-2004.
5. Blanchard TL, Thompson JA, Brinsko SP, et al: Mating mares on foal heat: a five –year retrospective study. Proc Am Assoc Equine Practit 2004. p. 525-530.

Reconstructive surgical procedures to enhance mare fertility

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Abstract

Surgical techniques for repair of pneumovagina, urovagina, perineal lacerations, recto-vaginal fistulas, and cervical lacerations are described.

Keywords: Pneumovagina, urovagina, perineal laceration, recto-vaginal fistula, cervical laceration

Preparation for standing perineal surgery

The mare is restrained in standing stocks, and the tail is wrapped and elevated. Ensure your have a quick release knot on the tail in case the mare does go down in the stocks. The rectum is evacuated and the perineum, vagina, and rectum are cleaned depending on the surgical procedure. An epidural anesthesia may be performed for some procedures (see below). Appropriate instruments (long- handled instruments) should be available. A headlight is very helpful for this type of procedure. Often the mare's foal is present, so you may have to sedate both the mare and foal during the procedure. The foal can also be confined in a small pen next to the mare.

Methods of providing anesthesia for surgery

- Local infiltration anesthesia
- Epidural anesthesia
    - Local anesthetics
        - Lidocaine 2% (20 mg/mL)
        - Mepivacaine 2 % (20 mg/mL)
        - Depress axonal conduction in sympathetic, sensory, motor fibers
        - May cause motor weakness and collapse in hind legs
        - Onset in 15 minutes and duration of two to four hours
    - Alpha-2 adrenergic agonists
        - Inhibits release of a spinal neurotransmitter important in pain perception
        - Caudal analgesia, no extra-spinal effects, and motor tone maintained
        - Slower onset (30-45 min), longer duration of analgesia (2.5-3.5 hr)
        - Xylazine - 100 mg/mL
- Epidural technique:
    - A 1.5" x 18 ga needle is used
    - Wearing sterile gloves, place needle into the space between S-5/Cy-1 or Cy-1/Cy-2 after the area has been clipped and aseptically prepared
    - The solution should be injected with no resistance
    - "Hanging-drop" technique

Pneumovagina

Pneumovagina is commonly treated by performing an episioplasty or Caslick's procedure. In certain cases where there is atrophy or a laceration of the perineal body, a perineal body reconstruction procedure (Gadd technique) can be performed. A Gadd technique involves closure of the dorsal vulva after two large triangular flaps of mucosa, which connect dorsally, have been removed. Removal of the mucosal flaps can be performed using local anesthesia. The defect created by removing the mucosa is then sutured closed to appose the dorsal aspect of the vulva. The skin of the vulva is closed as with the Caslick's procedure. This procedure can be performed easily and efficiently and carries a very good prognosis.

Urovagina/urine pooling

This condition is primarily observed in older multiparous mares. Urovagina results from laxity of ovarian and/or pelvic supporting ligaments due to age and repeated pregnancies. Urovagina can also result from poor conformation and body condition. The sagging of the uterus into the abdomen will pull the vagina with it. The urethral orifice is pulled forward and when urine is voided, some gravitates cranially, causing vaginitis, cervicitis, endometritis, and infertility. Some mares may initially pool urine in the vagina intermittently and careful conservative management of breeding may be occasionally successful in these mares. Clinical signs include

infertility, urine scalding, and often these mares have an odor like urine or ammonia. The diagnosis is based on clinical signs and vaginoscopy. If the urine pooling cannot be controlled medically, then surgical intervention is recommend. In some cases an endometrial biopsy is recommended prior to surgery to evaluate the endometrial tissue.

Surgical correction of urine pooling is one of the most frustrating reproductive surgeries. The urethroplasty procedure may be performed without problems, but the outcome is difficult to predict. Surgical repair failure can result in fistula development. Fistula development results in continued urine pooling and surgical closure of the fistula can be difficult to achieve. In many cases the entire procedure should be repeated.

Mares are sedated and the urinary bladder can be catheterized. Epidural anesthesia is performed as described earlier. The rectum is evacuated and the vulva and vagina are cleaned using a diluted iodine solution. The choice of urethroplasty technique generally depends on the surgeon's preference and potentially the mare's conformation. The general urethroplasty technique involves creating a mucosal tunnel from the urethral orifice to near the mucocutaneous junction. Several techniques which create a mucosal tunnel have been described.[1-4]

The author tries to perform a technique that is a combination of the Brown and McKinnon techniques. The tunnel is sutured in multiple layers,[3] but the tunnel is made by creating the mucosal shelves for the tunnel more dorsally within the vestibule.[4] After completion of the tunnel it is very important to maintain a urinary catheter. In the author's opinion this is very important for the success of the procedure. Generally, a Foley catheter is used for 14 to 21 days and the catheter is changed every four to five days. The catheter should be flushed daily with an antibiotic solution (10 mL of gentomycin in a liter of saline) to prevent crystal blockage of the catheter. In the author's experience, the cases where the mare cannot maintain the urinary catheter are more susceptible to fistula development. Mares are discharged the same day or the following day on systemic antibiotics and nonsteroidal anti-inflammatory agents.

Another technique involves pulling the entire vestibule caudally by transecting the perineal body horizontally.[5] The new position of the vulva is sutured in place at the site of the perineal body transection to maintain the new position of the vestibule. This creates a shelf, which often becomes contaminated and collects fecal material, but this does not represent a significant long-term problem. This technique has been described for alleviating pneumovagina as well.

The main complication following urethroplasty is the risk of fistula formation and continued pooling of urine. Other complications include signs of colic and straining to urinate. Parturition can disrupt a previous urethroplasty technique and a second surgery may be required to reestablish the urethral extension.

Recto-vaginal lacerations

Perineal lacerations generally occur during foaling in primiparous mares because of the forceful expulsion of the foal. The extent of the damage varies and can be classified into three degrees. First degree perineal lacerations involve the mucosa of the dorsal vestibule and vulva. These lacerations are generally treated by performing an episioplasty or Caslick's. Second degree lacerations involve the submucosa and muscularis of the vagina, vestibule and perineal body. The laceration does not involve rectal mucosa or anal sphincter. Most of these lacerations heal satisfactorily by second intention. In cases with significant damage, the result may be the development of pneumovagina. As with first degree lacerations, an episioplasty may be all that is needed to correct the pneumovagina. In cases with significant disruption of the perineal body, a Gadd technique may be required. Third degree perineal lacerations are the most serious due to fecal contamination of vestibule, vagina, and uterus resulting in infertility. The laceration involves penetration through the rectal-vaginal shelf and musculature of the vagina, vestibule and rectum. Repair is usually delayed until the necrotic tissue has sloughed and the wound has healed by second intention. In most cases, three to four weeks postpartum is adequate. However the repair should be done as soon as possible to prevent long-term fecal contamination of the uterus. Preoperative preparation and postoperative management involves softening the feces to minimize tension on the surgical repair. If available, mares should be placed on lush pasture prior to surgery until feces are soft and then kept on lush pasture for at least two weeks after surgery. Other methods of softening the feces include substituting hay and grain diet with a pelleted ration three to four days before surgery, and adding mineral oil and magnesium sulfate. Perioperative antibiotics and nonsteroidal anti-inflammatory agents are administered.

Epidural anesthesia is generally used on mares with third degree perineal lacerations and rectal vaginal fistulas. Caudal epidural anesthesia (S6-Cy1 or Cy1-Cy2) is performed, using a combination of 100 mg xylazine hydrochloride and 40 mg 2% mepivacaine hydrochloride, diluted with sterile saline solution to make a final injection volume of seven to ten mL. The rectum, vulva and vagina are cleaned of gross contamination using a dilute iodine solution. The method of repair can involve one or two stages. A one-stage repair involves complete closure of the rectal-vaginal shelf and perineal body. In a two-stage repair, the rectal-vaginal shelf is repaired

initially, and then at least three to four weeks later, the perineal body is repaired to complete the second stage. Generally the second stage can be performed using local anesthesia.

Closure of the rectal-vaginal shelf can be performed using either of two methods. In both methods, an incision is made to create a rectal shelf and a vaginal shelf on both the left and right sides of the defect. The incision is generally through the demarcation of the rectum and vagina, but should extend more into the vagina as the incision courses caudally. This helps eliminate tension on the final repair. Shelves are created at the cranial aspect of the defect as well. It is important to extend cranially about 2 to 4 cm when creating the shelves in the cranial aspect of the defect, and the shelves on the sides should be undermined laterally so that there is minimal tension on the closure. The shelves can be closed using a simple continuous pattern in a cranial to caudal direction. The vaginal side is closed first in 3 to 4 layers and then followed by the rectal side. Another method involves moving in a cranial to caudal direction using an interrupted six-bite pattern to close both the vaginal and rectal layers together. Post-operatively, mares are treated with broad-spectrum antibiotics and nonsteroidal anti-inflammatory agents. It is important to keep the feces soft so that the surgical repair does not dehisce. Mineral oil may have to be given daily to help keep the feces soft in some cases. The main complication following surgery is the potential for fistula formation or dehiscence along the suture line. The prognosis is generally very good to excellent following repair, but this may be due to the advantages of the lush grass in our area. Recurrence the following year is a possibility, therefore special attention during parturition is important.

Recto-vaginal fistula

Recto-vaginal fistulas occur when the foal's foot penetrates through the rectal-vaginal shelf, but does not rip all the way out through the anal sphincters and perineal body. Generally the fistula is caudal to the remnant of the hymen (within the vestibule), but in rare cases can be cranial to it (within the vagina). Some fistulas can heal spontaneously and not require surgical repair. The fistula is generally large after parturition, but heals and contracts to be only a few centimeters in diameter in three to four weeks. Fistulas can also occur with partial dehiscence of surgically repaired third degree rectal vaginal tear. Surgical repair is required to restore the mare's fertility. Perioperative management is the same as for third degree rectal vaginal tears. Methods of surgical repair include: converting the fistula to a third degree perineal laceration, direct closure through the vagina, direct closure through the rectum, or transecting the perineal body horizontally to expose the fistula for repair. I personally prefer to transect the perineal body horizontally and then to transect the fistula to create a rectal defect and a vaginal defect. The rectal defect is closed first in one layer by everting the edges of the fistula into the rectum. Closure of the rectal side first ensures a stronger and easier closure. The vaginal defect is then closed in two to four layers using an inverting pattern. Absorbable suture material is used for closure of both the rectal and vaginal defects. The dead space between the rectal and vaginal closures is left open to heal by second intention. The skin is closed on the left and right sides of the perineal incision but the center is left open to provide drainage. Post-operatively, mares are treated with broad-spectrum antibiotics and nonsteroidal anti-inflammatory agents. The prognosis is generally very good to excellent following repair, but this may be again due to the advantages of the lush grass in our area. Recurrence the following year is a possibility, therefore special attention during parturition is important.

Cervical lacerations

Cervical tears can be a potential cause of infertility in mares. Late-term abortion or a difficult dystocia have been associated with the development of cervical tears. The inability of the cervix to close properly will potentially result in a chronic uterine infection. Digital examination of the cervix is very valuable for evaluation, and the laceration may not be evident on visual examination. I like to evaluate the cervix during diestrus, because it is easier to identify the injury within the fibromuscular layer. Surgical repair of large cervical lacerations is needed to restore the future fertility of a mare. Surgical repair should be delayed until three to four weeks after parturition. I generally prefer to perform the surgery during diestrus. This surgery requires long instruments, a good head light and a dorsal speculum.

In most cases, cervical lacerations are repaired with the mare standing in stocks using epidural anesthesia. Tears that involve the dorsal aspect of the cervix are easier to repair in standing mares. Tears that involve the ventral aspect of the cervix are generally more difficult to repair with the mare in a standing position. The cervix is retracted caudally using either stay sutures or cervical retractors. I prefer stay sutures placed on each side of the defect. The edges of the defect are debrided using long-handled scissors and closed using a two- to three-layer closure. The goal of the surgical repair is to create a cervical os that one finger can be passed through easily.

Tears that involve the ventral aspect of the cervix are generally more difficult to repair because of lack of visibility of this area. In some cases, the temperament of the mare or a small vestibule (limiting visualization) makes standing surgical repair difficult. If the cervical repair is not successful, the mare will continue to be infertile.

Secure closure of ventral cervical lacerations can be achieved by positioning the mare into a Trendelenburg position. The hind quarters of the mare are elevated using a hoist system. These tears can be easily and efficiently repaired in this position.

Post-operatively, mares are treated with broad-spectrum antibiotics and nonsteroidal anti-inflammatory agents. Recommendations are made to digitally apply antiseptic or antibiotic ointments to the cervix every couple days for ten to14 days to ensure the cervical os remains patent. Prognosis is generally good but this depends on the size and location of the cervical tear. The mare become pregnant after a cervical repair, but the mare still may not be able to carry the foal to term. The cervical repair may not be able to withstand the weight of the pregnancy which can result in the development of placentitis in the last trimester.

References

1. Monin T: Vaginoplasty: a surgical treatment for urine pooling in the horse. Proc Annu Conv Am Assoc Equine Pract 1972. p. 99.
2. Shires M: A practical and simple surgical technique for repair of urine pooling in the mare. Proc Annu Conv Am Assoc Equine Pract 1986. p. 51.
3. Brown MP, Colahan PT, Hawkins DL: Urethral extension for treatment of urine pooling in mares. J Am Vet Med Assoc 1978;173:1005-1007.
4. McKinnon A: A urethral extension technique to correct urine pooling (vesicovaginal reflux) in mares. J Am Vet Med Assoc 1988;192:647-650.
5. Pouret EJ: Surgical technique for correction of pneumo- and arovagina. Equine Vet J 1982;14:249-250.

Breeding shed safety

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Abstract

Safety in the breeding shed should be considered at all times, both for personnel and breeding stock. Implementation of practical means for reducing the risk of injury, including animal restraint, breeding shed design, and handler training and protective clothing, should be an important part of not only every breeding operation, but also in the training of veterinary students[1] who plan to work with horses. Precautions taken to optimize safety will improve biosecurity as well.

Keywords: Stallion, semen collection, restraint, safety

Introduction

Working with horses poses an inherent risk of serious injury for even the most experienced horseman. The Equestrian Medical Safety Association (EMSA) analyzes data provided by the National Electronic Injury Surveillance Survey (NEISS) for horse-related injuries seen in hundreds of emergency rooms (ER) across the United States on an annual basis. Results from this analysis in 2007[1] showed that of 78,279 injuries seen, the most common injuries reported in this study were fractures (28.5%), contusions and abrasions (28.3%) and strain/sprains (14.5%). Less common injuries included internal injury (8.1%), lacerations (5.7%), concussions (4.6%), dislocations (1.9%) and hematomas (1.2%). The most common sites of injury were the lower trunk (19.6%), head (15.0%), upper trunk (13.4%), shoulder (8.2%), and wrist (6.8%). In this study, fatalities comprised less than 0.1% of the injuries seen by ER personnel. Other researchers have reported that the majority of horse-related deaths are not performance-related, but behavioral in nature[2] and occur while a person is unmounted.

In this regard, it is estimated that 20-34% of horse-related injuries are the result of an unmounted accident, as opposed to injuries that occur while horseback riding.[3-5] Although the sites of injury to unmounted patients was similar to mounted patients (lower extremity (34.7%), upper extremity (25.%) and head (20.2%)[3]), the most common sites injury of the unmounted patient were found to be in far greater proportion of total injury sties when compared to those of the mounted patient. The most frequent mechanism of injury to the unmounted patient was a kick or strike by a horse (11.2%), followed by being stepped on (6.1%) and pushed into, jerked or knocked down (3.0%).[4] Other less common circumstances that lead to unmounted injuries include bites, horses spooking, striking an object, catching a body part, being hit by a horse's head, and being hit by a falling horse.

The equine breeding shed, without a doubt, requires highly conscientious and experienced horse handlers for smooth operation. The primary objective in the shed is the transfer of stallion semen, either by natural cover or collection. Sometimes overlooked is the importance of safety in the shed, for both personnel and horses. Interestingly, many of the precautions taken to ensure safety with breeding stock not only protects horses and people, but also contributes to optimizing the quality of the ejaculate. Furthermore, many precautions also ensure a greater degree of biosecurity in the process as well.

There are many ways on a breeding farm to optimize safety, from the design of the shed to restraint of the mare and stallion,[6] to the quality of a phantom, to training and attire of personnel. This paper presents many practical means for optimizing safety in the equine breeding shed.

The breeding shed-physical layout

The design of a breeding shed needs to address not only its physical layout but also its proximity to the stallion stalls and laboratory. In order to avoid the detrimental effects of UV light and temperature changes on semen quality, semen collection should take place within a short distance of the laboratory where semen evaluation and processing will take place. A roof, high enough to accommodate a large stallion that rears, is necessary not only to protect the area from inclement weather, but also to keep sun from overheating the phantom or affecting the quality of collected semen.

The proximity of stallion housing also needs to be considered. There are many advantages to housing a stallion close to the shed, for the distance that an excited, less trained stallion travels on his way to shed is minimized. Housing that is close to the shed may be an advantage as well to a young or

inexperienced stallion, or one with low libido, for the stallion is able to at least have the auditory, if not the visual stimuli of the breeding shed.

On the other hand, a long walk to the breeding shed may be optimal for stimulating a quieter stallion with low libido. However, for more fractious stallions, a long distance can turn into a threat to both the handler and the stallion. Furthermore, for stallions that produce dilute semen, the excitement and maintenance of an erection during a long walk to the shed will likely result in a semen sample that is more dilute from prolonged accessory gland stimulation and resultant excessive seminal plasma. This dilemma often poses a problem during the breeding season, regardless of where a stallion is housed, even if arriving in a trailer from a site off the farm; the stallions anticipate collection and are excited longer than desired for optimal semen collection.

The size of the breeding shed should allow ample area for movement of personnel and both a tease mare and stallion. A wash area, ideally padded, can be located within the shed or just adjacent to it. Locating the wash area within the shed holds the advantage of stimulation of the stallion to attain and maintain an erection. However, for the unruly stallion, a wash area within the shed may pose unnecessary stimulation and he may become more difficult to handle. He may be more easily washed in a smaller enclosed space just before entering the shed. A seasoned, well-managed stallion can be washed in a large open space.

Many successful breeding operations have designed an adequate and safe shed that is no larger than a 12 ft X12 ft stall. Regardless of the size of a breeding area, there should be numerous escape routes for personnel, as well as the tease mare. These escape channels can be gates that open, sliding doors, padded walls or narrow spaces left between fence rails. As well, the entire property should be secured by fencing and gates should any of the breeding horses become loose during breeding or collection.

Mare teasing

The size of the mare teasing area should be at least as large as a set of stocks plus a full diameter equal to a stallion's length. A teasing chute is an alternative and can have the advantage of providing a double function as a multiple mare teasing area and a mare breeding/ collection area. A mare can be positioned with her chest against the wall and her head over the wall, secured by a handler. Also, a single stall in the breeding shed can be used as a mare teasing area or can house a stallion that perhaps requires mares to approach him for arousal. Regardless of the mare teasing structure, padding with either foam or rubber protects the breeding stock from injury.

Some mare teasing areas permit a stallion's head to have direct physical access to the mare, thus optimizing tactile sensation for arousal. This set-up is ideal for collection of stallions "on the ground" for the stallion can undergo teasing and lean into the solid wall while semen is collected. A teasing wall can also be designed to protect the mare handler during natural cover because the handler can be positioned on the opposite side of the wall from the mare and stallion. The disadvantage to such a system is that the stallion or mare may attempt to mount the "wall" and become injured or even stuck. If metal bars are used to separate a stallion from the tease mare, the bars must spaced closely together so that the stallion cannot hang a foot or a shoe between the bars if he strikes or kicks.

A teasing mare may also be restrained by a handler and positioned beside a phantom. There must be adequate space not only for positioning of the mare's body, but for exiting the breeding shed rapidly should the need arise.

Phantom

The phantom should be located in close proximity to the teasing area, ideally within a few steps forward, backwards or to the side. This system works well for training stallions to the phantom as well as for easily managed stallions that are habituated to the collection protocol.

There are numerous commercial phantoms available. They vary somewhat in shape, size and accoutrements, but they are all a solid structure wrapped in thick foam and covered with durable material. Variability in both height and slope may accommodate different sizes of stallions. Ideally, the outer phantom cover should be durable, nonabrasive, disinfectable and provide adequate traction for the front legs of the stallion. Seams should be sewn on the inside of the cover so that neither the front legs nor the penis of the stallion is abraded. Leather phantom covers are durable and provide traction but cannot be disinfected adequately. Wrapping the mounting/collection end of the phantom in plastic wrap between stallions will minimize the possibility of venereal transmission of organisms. However, it will not protect against oronasal transfer of organisms at the head of the phantom. A durable, slightly roughened, material

called olefin (Herculon®, Hercules, Inc, Wilmington, DE) is an ideal material for a phantom cover because it is less expensive than leather and it can be scrubbed with detergent and rinsed with alcohol between stallion collections.

Some managers prefer a cut-away mounting end of the phantom to better secure the artificial vagina (AV) during collection. This system works well unless collecting a stallion that has a penile deviation to the opposite side. Phantoms are also available with removable artificial vaginas that fit into the mounting end. Although advantageous in that a single person can both handle and collect a stallion that has been trained to such a device, this type of phantom may prove unsafe for amateur stallions that move rapidly around the rear end of the phantom while mounted. Stallions have been injured by these phantoms with a built-in AV either from catching a foot in the latched opening for the AV or lacerating the penis when it is inadvertently thrust between the AV and the metal interior of the phantom.

For facilities that collect semen from a wide variety of stallions, a basic solid phantom covered in olefin or leather is advantageous. As well, a washable, faux fur phantom cover can be placed over the phantom for collection for the occasional stallion that suffers abrasion of the medial carpi while gripping the phantom during collection. This superficial injury may cause a stallion to be reluctant to mount the phantom or work the AV. Stable wraps applied to protect the carpi are often ineffective during collection for they not only slip but may deter the stallion from bending his forelegs adequately.

Footing

There are many materials available for footing in a breeding shed. The most important considerations in choosing the flooring are cost, resistance to slipping, particle dust and biosecurity. Gravel, sand, compacted dirt and hog fuel (a coarse mix of chips of bark and wood fiber) are examples of inexpensive materials that are not slippery. However, particles are readily kicked up as the stallion moves, often leaving traces of particulate matter on the penis, which in turn can lacerate the penis and make the stallion reluctant to breed. If these materials are used, spraying the surface before collection can reduce the amount of particulate matter. Unfortunately mud puddles develop from spraying and dirt and debris may be splashed on the penis as well. The use of large particle (3/4 -) gravel has been used successfully in many breeding operations. It tolerates hosing, provides good traction, and it is not so large that barefoot stallions may become foot sore.

None of the materials discussed above confer adequate biosecurity, for they cannot be disinfected. The use of rubber mats, rubber bricks, or sprayed composite flooring has the advantage of permitting good disinfection. However, traction is sometimes compromised and they are expensive materials. As an alternative, the placement of a woven coconut or plastic grass mat at the mounting of the phantom can provide good traction but again, cannot be disinfected easily.

The author has found that recycled rubber square tiles or bricks (Pavesafe®, Ecore International. Lancaster, PA), or a comparable product, are ideal for the breeding shed floor in meeting the criteria for horse safety and biosecurity. The surface provides ample traction for the less experienced or agitated stallion and can be easily disinfected.

Lastly, adequate drainage must be considered in designing a breeding shed. Whether washing the phantom, equipment, the perineal area of a mare or the stallion's penis, the water that collects on the ground may make the flooring slippery or form puddles that can splash debris on the stallion's penis.

Restraint-stallion

Although excellent articles have been written on appropriate handling and restraint of a stallion in the breeding shed,[7] there is no form of restraint that replaces adequate training in basic horsemanship. This training can be started and effective at any age as long as it is consistent and properly reinforced. It is, in the authors' opinion, far more challenging to retrain a stallion that has developed bad manners than to teach the expected behavior from the beginning of training. Although normal behavior permitted in the breeding shed is different than that expected on a show ground, the basic principles of respecting space and command of a handler are the same.

A stallion that does not respect these basic principles should be removed from the breeding shed and either taught or reminded of appropriate behavior in a place where the stimulus of breeding is not a distraction. Once the stallion is responsive, he is permitted to reenter the breeding shed.

The mantra that "less is more" is applicable to restraint of a stallion. Restraint that is appropriate for one stallion may be inappropriate for another. Each stallion must be regarded and evaluated individually. Some seasoned, well-mannered stallions require no further restraint in the breeding shed than

a properly fitted halter and cotton lead rope, or a lariat formed "war bridle". They yield to light pressure, which can be as little as a flick of the rope, a touch on the shoulder, or voice command. In fact, imposing any greater restraint on these stallions may serve to inhibit normal behavior in the breeding shed. Most stallions, however, require more restraint.

The most common restraint device used on a stallion is the stud chain. Made of heavy metal links, a stud chain can vary in length between 12 to 24 inches. The author prefers a stud chain that is not attached to a leather shank or rope so that it can be applied or removed easily. The application of the chain to sensitive areas of the horse's head and mouth will vary in severity depending on the site. In general, the areas of minimal pressure to maximal pressure on which the stud chain is applied include over the nose, around the nose, under the chin, through the commissures of the mouth (like a bit), and against the gum of the maxillary incisors (called a lip chain).

Some owners request that handlers refrain from placing a chain over the nose to avoid bruising, scarring or laceration. In general, application of a stud chain beneath the mandible should be used judiciously and with caution because the mandible can be easily fractured with an excessive jerk of a heavy chain. As well, only the most experienced handlers should use a lip chain, for only light pressure need be applied, less one might inhibit normal breeding behavior of a stallion or cause injury to the maxillary gum.

A variety of attachable bits can be snapped to the halter as well. Many breeding sheds use a stallion bridle and bit for restraint, especially if the stallion is accustomed to being worked in a bridle. At many European studfarms, young colts are worked daily in bridles, hand-driven in long lines. These long reins can be extremely effective in properly positioning and handling a stallion in the shed and possibly offer the ultimate control over the direction of his entire body.

When introduced to a new breeding stallion, it is difficult to initially determine the degree of restraint that will be optimal for that stallion in the shed. The stallion may be readily responsive to voice command and a light touch in the stall or on the way to the shed, and then his behavior may become far more aggressive once the shed is entered. Sometimes it is beneficial to place a chain appropriately on the halter but attach the lead rope, not to the chain itself, but to the halter; it can be readily transferred to the chain if necessary. Or the handler can carry two ropes, one attached to the chain to apply pressure if necessary and the other attached to the halter if the stallion behavior is dampened with pressure of the chain.

Another restraint device is called a stallion halter or iron halter. This device is a leather or nylon halter with a heavy iron noseband. A quick jerk on the noseband causes sharp pain; often stallions will respond quickly towards submission. Again judicious use of such a device is advised for, following improper use of this device, a stallion may then show extreme reluctance to exhibit normal libido and behavior. It can, however, be used effectively on stallions if their attention is diverted elsewhere or they do not heed the pressure of a stud chain.

A muzzle, made of iron or nylon, may be used to protect both mares and the handler from a stallion that bites. One of the disadvantages of the more common iron muzzle is that the handler can be inadvertently hit with the muzzle if the stallion swings his head. Thus a muzzle made of nylon may be a safer choice.

Some young stallions are easily distracted or frightened during the early training sessions in the shed. Blinkers of various sizes are easily applied over a halter and may help to focus the stallion's attention. There is a report in the literature that describes placing a blindfold on a stallion that persistently rushed mares in attempt to breed.[8] The mares were startled and kicked at the stallion. The stallion's behavior was unchanged by the mare's reaction so a blindfold was put over the eyes of the stallion. Apparently, the blindfolded stallion was taught to walk quietly to the rear of the mare and eventually mount her. Over time, the trainers could remove the blindfold and the stallion continued to mount the mares more calmly. Caution, however, must be exercised with a blindfolded stallion that might panic and run over the handlers.

Other tools used for stallions that rush a mare or the phantom include anything that will divert his attention away from his area of interest. Some stallions may respond to the mere presence of a whip and refrain from charging. A colorful plastic bat that makes great noise upon contact with the stallion's shoulder or chest is often very effective in diverting a stallion's attention. A plastic sleeve tied to the end of a dressage whip may also be effective. Because stallions are likely to become desensitized to these diversions, appropriate training must be applied at the same time that these tools are used.

Restraint-mare

Adequate protection of the mare and from the stallion must be addressed as well. A mare in good standing heat makes an ideal tease mare. Housed in padded stocks for teasing, she is protected from the stallion and he is protected from her. Often though, the mare is not restrained within stocks, for she is either acting as a jump mare for semen collection or being bred.

The mare's restraint is equally important so that the stallion and personnel are not in jeopardy. Hobbles can be applied to her rear legs to minimize the impact of a kick. Hobbles, however, are often more of a liability than an asset, for they can release unexpectedly, or tangle the legs of a panicked mare or inexperienced stallion. A quick release mechanism of the hobbles must be readily accessible and easy to use should the breeding go awry. Felt or leather booties can be used to cover the rear hooves of the mare to lessen the impact of a kick as well.

Many of the restraint devices described for the stallion may be used on the mare. In addition, the application of a twitch to the upper lip of the mare may be useful in immobilization of the mare. There are some disadvantages to using a twitch on the mare, however. First, it may require one more person in the breeding area that is either in the way of others or at risk themselves. One can use a jobar twitch that hooks through the halter, requiring only one person to handle the mare, but this twitch can be difficult to remove quickly. Second, a handler may want a mare that is more mobile, either to stimulate the stallion effectively or to move quickly out of harm's way if the breeding does not go as planned, and a twitch may render a mare immobile.

Occasionally a mare may require tranquilization to be bred or serve as a mount. This option should be considered as a last resort because it is challenging to balance the ideal amount of chemical restraint for an individual mare. If there is not enough sedation, the mare may kick or strike without warning. If there is too much sedation, she may be ataxic and unable to support the stallion's body. Moreover, a sedated mare may no longer provide adequate stimulus for the stallion.

Lastly, to protect the mare from the stallion's bite, a shroud can be worn around the neck of the mare. Many stallions will grasp the shroud within their teeth either during teasing or while mounted.

Personnel

The importance of the person who handles the stallion to the entire operation of the breeding shed cannot be overemphasized. The stallion handler is ultimately responsible for directing the stallion and other personnel as well as the mare within the shed. They should have not only extensive horse experience, but experience in handling stallions. Restraint to all breeding stock must be applied in an effective and humane way with appropriate timing of reprimand and reward.

Many injuries to breeding shed attendants and horse handlers can be readily prevented with appropriate attire. Helmets should be a requirement. The most common helmets used are ASTM-certified riding helmets, but a motorcycle or hockey helmet with a face/mouth guard may yield superior protection. Bicycle helmets or construction hard hats offer less protection. Regardless of the headgear, one must ensure that hearing is not impeded by the helmet or padding.

The use of heavy shoes and leather gloves protect the feet and hands of handlers. A leather jacket may lessen or prevent injury from a bite. Finally, a safety vest, such as those required for cross-country riding, may protect against upper body injury.

Communication is equally important to all the above means of restraint and safety. Communication between personnel before, during and after collection is paramount. Diversions, such as unnecessary conversation or cell phone activity, may prove hazardous.

It is important for personnel to form a plan. One person should be in charge and that responsibility is usually designated to the stallion handler. For purposes of not only liability, but also safety, personnel in the breeding shed should be employees of the veterinarian.

Some people that collect stallions have been trained to collect on the same side handler. Others prefer to collect on the opposite side. There are advantages and disadvantages to both situations. Having all personnel on the same side not only allows a stallion an escape route on the opposite side without encountering a person or the tease mare, but it permits all personnel to see what is happening and know the location of all involved. The disadvantage is that they may trip over each other. This will not happen if the semen collector is on the opposite side of the stallion and mare handlers. However, the collector is then at risk, for they are more likely to be kicked by the stallion or mare.

All horses are unpredictable and therefore an evacuation plan should be discussed in advance. Stallions can get loose. They can lose consciousness while mounted. The teasing and breeding may simply

not go as planned and horses and personnel must be quickly evacuated until a new plan is developed. Although it is impossible to plan for all scenarios, agile and experienced personnel will optimize safety.

Conclusion

Safety in the breeding shed must incorporate, in order of importance, the safety of personnel, followed by the safety of breeding stock,[9] followed by biosecurity, and lastly, success of breeding or collection of semen and resultant quality of the ejaculate. It is important, as well, to ensure that training for safety in the breeding shed is incorporated into the veterinary school curriculum.[10]

Each stallion should be evaluated individually and appropriate steps must be taken to ensure breeding shed safety. Optimal preparation of equipment, personnel and horses will lend a greater degree of security and more positive experience for all involved.

References

1. Bixby-Hammett D: Horse related hospital emergency room admissions, 2008. Equestrian Medical Safety Assoc Arch 2009;12:1-14.
2. Houpt KA, Mills DS: Why horse behavior is important to the equine clinician. Equine Vet J 2006;38:386-387.
3. Thomas KE, Annest JL, Gilchrist J, et al: Non-fatal horse related injuries treated in emergency departments in the United States, 2001-2003. Br J Sports Med 2006;40:619-626.
4. Landercasper J, Cogbill TH, Stutt PJ, et al: Trauma and the veterinarian. Am Medical Equestrian Assoc 2000;9:2.
5. Erkal S, Gerberich SG, Ryan AD et al: Horse-related injuries among agricultural household members: Regional Rural Injury Study II. J Rural Health 2009;25:420-427.
6. Merkt H, Klug E: Safety precautions during semen collections from stallions. Dtsch Tieraztl Wochenschr 1989;96:491-493.
7. Varner DD: Handling the breeding stallion. Proc Am Assoc Equine Pract 2005; p. 498-505.
8. Richardson GF, Wenkoff MS: Semen collection from a stallion using a dummy mount. Can Vet J 1976;17:177-180.
9. Grandin T: Safe handling of large animals. Occup Med 1999;14:195-212.
10. Chapman HM, Taylor EG, Buddle JR et al: Student training in large-animal handling at the School of Veterinary and Biomedical Sciences, Murdoch University, Australia. J Vet Med Educ 2007;34:576-582.

Sperm transport, elimination and endometritis

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Abstract

Sperm transport in the mare's reproductive tract is a rapid event, stimulated by uterine contractions. Recent observations suggest that seminal plasma also plays an important role in sperm transport and elimination from the uterus. Only a small portion of spermatozoa in an ejaculate reach the oviduct. The remainder of spermatozoa are eliminated through breeding-induced uterine contractions and an influx of leukocytes in response to breeding. The breeding-induced inflammation serves to eliminate dead spermatozoa from the uterus while seminal plasma proteins protect viable spermatozoa from this form of elimination. The transient inflammation typically resolves within 24-36 hours. Mares that fail to resolve the inflammation within this time develop a persistent breeding-induced endometritis, which can interfere with pregnancy. Treatment for persistent breeding-induced endometritis should be focused on the elimination of inflammatory products and fluid from the uterus, and restoring an environment that is compatible with the survival of an embryo.

Keywords: Equine, spermatozoa, endometritis, sperm transport, sperm elimination

Introduction

In order to successfully fertilize an ovum, spermatozoa need to be transported to a site within the female reproductive tract where they can be stored until ovulation occurs, be further transported to the site of fertilization, and undergo biochemical changes that enable them to bind to and penetrate the oocyte. In addition to motility and morphology of the spermatozoa, factors such as the presence and localization of sperm membrane proteins, biochemical properties of seminal plasma, uterine contractility, and the micro-environment of the female reproductive tract might also be essential components for successful fertilization to take place. For example, the integrity of the macromolecules of the sperm membrane may be an important component of sperm longevity.[1-2] Uterine motility during estrus and in response to semen are likely to be important factors for sperm transport.[3-4] Sperm transport and elimination from the uterus may be affected by seminal constituents;[5-10] and membrane biochemistry is important for sperm capacitation, acrosome reaction and the final steps of fertilization.[11] All these factors may affect transport and survival of spermatozoa in the female reproductive tract. Studies on sperm physiology *in vivo* are difficult, however, since an ejaculate contains many subpopulations of spermatozoa with heterogeneous characteristics. Breeding and ovulation do not necessarily happen at exactly the same time. Heterogeneity within an ejaculate may therefore, be required to ensure that fertile spermatozoa are present for an extended time in the female tract.[12] At any given time, it is not possible to distinguish which spermatozoa are capable of fertilization from those that are not yet competent to fertilize an egg, or from those that may never be capable of fertilization.

The purpose of this review is to summarize current literature and observations in the author's laboratory on sperm transport, elimination from the female tract, and the role of breeding-induced endometritis.

Sperm transport

Spermatozoa are deposited directly into the uterus in the mare. The time for equine spermatozoa to reach the oviduct following mating is relatively short. Sperm have been identified in the oviduct within one hour after artificial insemination (AI), and sperm transport is believed to be completed within four hours after breeding.[13-17] Spermatozoa have to overcome many obstacles before they reach the oviducts. Less than 1% of inseminated spermatozoa are successfully transported to the oviduct after AI.[17,18] In addition to a rapid retrograde loss of spermatozoa through the cervix immediately after breeding, the utero-tubal junction (UTJ) appears to serve as the major barrier for spermatozoa to reach the fertilization site in the oviductal ampulla in the mare.[17] It has been suggested that prostaglandin E (PGE) may facilitate transport of spermatozoa through the UTJ.[8] PGE appears to exert a selective effect on the longitudinal muscle layer of the oviduct.[19-20] This results in an increased diameter of the lumen, which facilitates transport through the oviduct. The addition of PGE to semen resulted in an increased number of spermatozoa reaching the oviduct in one study.[8] In addition, fertility was improved in equines and humans[21,22] when PGE was added to semen. However, these results could not be repeated in a subsequent study.[23] Once in the oviduct, spermatozoa need to be stored without losing their ability to fertilize. It is believed that fertile spermatozoa are sequestered at a specific storage site in the oviduct until ovulation occurs. The caudal isthmus is the site of sperm storage in most species. The release of spermatozoa from their storage site is influenced by the time of ovulation, but the mechanism for this is not fully understood.[24]

Sperm transport and survival of spermatozoa in the female reproductive tract has been studied in fertile and subfertile horses.[17] Differences were found between fertile and subfertile stallions, and between fertile and infertile mares .[17] The authors found that the total number of spermatozoa that reach the oviduct at four hours after insemination with fresh extended semen was significantly greater from fertile stallions compared to subfertile stallions. In addition, a greater fraction of motile and morphologically normal spermatozoa were found in the oviducts of mares inseminated with semen from fertile compared to subfertile stallions. The authors also found differences in sperm transport among fertile and subfertile mares. When mares were inseminated with semen from normal fertile stallions, more spermatozoa of greater motility were observed in the caudal isthmus of reproductively normal mares, compared to subfertile mares. It was not clear whether the lower sperm number in the isthmus of susceptible mares was caused by dysfunctional sperm transport to the oviduct or impaired attachment of spermatozoa to the isthmic epithelium.

Although the motility of spermatozoa may contribute to transport through the female reproductive tract, contractions of the myometrium and myosalpinx may be more important in regulating the transport of spermatozoa to the site of fertilization.[3,8] Breeding stimulates contractions in the uterus as well as in the oviductal smooth muscle layers.[4,25] Using electromyography (EMG), uterine activity was recorded in mares following insemination with fresh extended semen.[4] The authors observed an immediate increase in the myoelectrical activity that lasted for 0.5 hours following insemination. The mechanism for this was not determined, but it has been shown that pituitary oxytocin is released in response to teasing of estrous mares to a stallion.[26] In addition, sexual stimulation and mating increase plasma concentrations of oxytocin in women, sows, ewes, and cows.[27-30] Equine seminal plasma contains prostaglandins and estrogens, which also could explain the myometrial response to breeding.[31] A second phase of myoelectrical activity was observed between four and >12 hours after AI in mares following insemination with fresh semen.[4] Since sperm transport is considered to be complete at four hours after breeding, uterine contractions during the second phase of myoelectrical activity are likely to be more important for sperm removal from the reproductive tract.[32]

Sperm elimination

Only a very small portions of ejaculated/inseminated spermatozoa migrate successfully to the site of fertilization. Elimination of semen through the vagina occurs within hours of insemination.[18] This rapid elimination of sperm from the uterus coincides with the increased myoelectrical activity following insemination and may therefore, be the result of uterine activity in response to insemination. A 25% loss of spermatozoa into the vagina was observed within a few hours after insemination with fresh semen.[18] The loss was higher following insemination with extended semen (74%) and with frozen/thawed semen (96%). The authors suggested cell membrane alteration and damage during handling of the semen as potential causes of these differences. Migration of sperm from frozen boar semen thawed in seminal plasma versus a glucose buffer was similar at one hour after insemination but the number of spermatozoa found in the oviduct at four hours after insemination was significantly higher when spermatozoa were thawed in seminal plasma.[33] The authors suggested that seminal plasma provided ways for the spermatozoa to survive for an extended time in the female reproductive tract. At the time of ejaculation, seminal plasma proteins are coating the spermatozoa, some of which protect the sperm cells from premature capacitation.[33] Seminal plasma proteins may also protect the spermatozoa against phagocytosis and elimination from the uterus.[6,8,34]

An influx of polymorphonuclear neutrophils (PMNs) has consistently been found in the equine uterus within a few hours of insemination.[35,36] Subsequent *in vitro* and *in vivo* studies suggested that both spermatozoa and seminal plasma play active roles in the inflammatory response to mating and that the PMNs contribute to the elimination of spermatozoa through phagocytosis of the sperm.[7,8,37,38] It is now generally accepted that a transient post-mating endometritis is a physiological reaction to breeding, with the purpose of eliminating excess spermatozoa, seminal products and contaminants from the uterus. This would aid the uterus in providing an environment that is compatible with survival of an embryo when it descends into the uterine lumen. Activated PMNs bind to spermatozoa in the uterine lumen and once phagocytosed, the spermatozoa are eliminated together with inflammatory fluid and products through uterine contractions. Since the inflammation is present in the uterus before sperm transport is completed, viable spermatozoa need to be protected from elimination. Research data from our laboratory demonstrate that components in seminal plasma protect viable but not dead sperm from PMN-phagocytosis.[39] The component in seminal plasma that is responsible for this protection was recently identified as cystein-rich secretory protein-3 (CRISP-3).[40] In addition to regulating sperm elimination, a function of seminal plasma may be to act as an inflammatory modulator in the uterus and at least in part, be responsible for the transient nature of a mating-induced inflammation.[7,38]

Endometritis

Most normal mares are fully capable of eliminating a uterine inflammation within five days following contamination of the uterus.[41] This ability is essential when the inflammation is associated with breeding. However, mares with compromised resistance to persistent uterine inflammation fail to resolve the breeding-induced endometritis in a timely fashion. If the mare's ability to clear an inflammation from the uterus is impaired, the inflammation will persist and turn into a pathological condition with lower fertility as a result.

Humoral, as well as cellular and physical components of the uterine defense system are involved in resistance to a persistent uterine inflammation. Within two hours of contamination (bacteria or semen), PMNs respond to a chemotactic signal resulting in a massive migration to the uterine lumen. Activated uterine PMNs phagocytize bacteria and spermatozoa, serving as an effective clearance of these antigens from the uterus. During the activation of PMNs, prostaglandin F2α (PGF) is released which causes myometrial contractions. This physical or mechanical uterine defense is a key factor in the prevention of persistent inflammation. In mares with a functional defense system, the majority of inflammatory products are cleared from the uterus within 24 to 36 hours of contamination. In contrast, susceptible mares fail to clear the uterus from contaminants and inflammatory products. An initial PMN-migration into the uterus also occurs in susceptible mares, and the freshly migrated PMNs are activated and fully functional. However, the physical clearance of inflammatory products is impaired in susceptible mares. This results in a sustained inflammatory response in the uterus with continuing migration of PMNs, rendering the environment incompatible with pregnancy if conception has taken place.

Using electromyography to study myometrial activity, it was observed that impaired uterine clearance in susceptible mares was associated with impaired myoelectrical activity.[42] It has also been shown that mares with delayed uterine clearance had an upregulation of inducible nitric oxide synthase (iNOS), resulting in an increased accumulation of nitric oxide in the uterus.[43] Nitric oxide mediates smooth muscle relaxation, which possibly could serve as an explanation for the impaired myoelectrical activity in response to an inflammation in susceptible mares.

Factors beside impaired myoelectrical activity that can predispose to delayed uterine clearance include anatomical abnormalities of the reproductive tract. Mares with delayed uterine clearance often suffer from a forward tilt of the uterus over the brim of the pelvis. This may be a contributing factor in abnormal accumulation of fluid and inflammatory products after breeding. A failure of the cervix to relax during estrus, or insufficient lymphatic drainage may also contribute to delayed clearance.[44]

Diagnostics

It may be difficult to identify susceptibility to persistent breeding-induced endometritis prior to breeding. Some mares have free fluid present in the uterine lumen prior to breeding, but most mares are not diagnosed until after they have been bred. Mares with a history of persistent breeding-induced endometritis are likely to develop this problem again. If susceptibility to persistent breeding-induced endometritis is suspected, the mare should be monitored closely by ultrasonography per rectum at six to 24 hours after breeding. If free fluid is present in the uterine lumen beyond six to12 hours after breeding, the mare should be considered to have persistent mating-induced endometritis.

Treatment

Management of mares susceptible to persistent breeding-induced endometritis should focus on assisting the uterus to physically clear contaminants and inflammatory products after breeding. Pre-existing conditions, such as uterine infections should be resolved before the mare is bred. Exposure to semen should be limited to a single breeding per cycle, if possible. This can be accomplished by closely monitoring follicular development in combination with the administration of an ovulatory agent. If intrauterine fluid accumulation is detected before the mare is bred, she can be treated with uterine lavage (using lactated Ringer's solution or a buffered saline solution) prior to breeding.[45] Physical clearance post-breeding can be assisted by the use of oxytocin or PGF treatment at four to eight hours after breeding. This protocol has been shown to aid in uterine clearance, resulting in improved pregnancy rates in susceptible mares.[46-50] Low doses of oxytocin (10-20 IU) appear to result in a more physiological myometrial contraction pattern and a more efficient clearance of intrauterine fluid, when compared to higher doses.[51,52] Care must be taken with regards to the timing of PGF treatment since PGF can interfere with normal development of a functional corpus luteum when administered within two days after ovulation.[53-57] Large-volume uterine lavage with a buffered saline solution at six to 24 hours after breeding will also effectively assist the uterus in clearing fluid and inflammatory products.[50] Fluid is infused into the uterine lumen and completely recovered in one to two liter increments until the recovered fluid is clear. There is a strong correlation between the clarity of recovered fluid and the presence of inflammatory cells in the fluid. Mares with persistent breeding-induced endometritis are not capable of effectively clearing the uterus from fluid, so care needs to be taken to make sure that all fluid is removed from the uterus. For this reason, many clinicians prefer to combine uterine lavage with

the use of oxytocin. Because sperm transport to the oviduct is completed within four hours after breeding, uterine lavage between six and 24 hours after breeding will not interfere with transport or fertility.[15] Manual dilation of the cervix, or local administration of PGE in mares with poor cervical dilation may help these mares to more effectively clear the fluid from the uterus.

The use of corticosteroids in mares with excessive inflammation in response to breeding has been suggested. In one study, the authors reported a positive effect on pregnancy rates following the administration of acetate 9-alpha-prednisolone (0.1 mg/kg) twice daily during estrus, starting when a follicle >35 mm was detected and ending when ovulation was confirmed.[58] In a more recent study, 50 mg of dexamethasone was administered to over 500 mares at the time of breeding.[59] Pregnancy rates were improved in mares with more than three risk factors for reproductive failure. These results are encouraging, but in contrast to a previous study that evaluated the effect of dexamethasone (40 ug/kg) in mares with experimentally induced endometritis.[60] These authors did not find a beneficial therapeutic effect of dexamethasone in reducing inflammation compared to antibiotics alone. Further research is apparently needed to clarify the mechanism of action for this treatment alternative. While the anti-inflammatory effect of corticosteroids may modulate the breeding-induced inflammation, it would also suppress the beneficial effect of endogenous prostaglandin on myometrial activity. It is, therefore, important to combine treatment with corticosteroids with oxytocin or PGF. A potential side effect of repeated dexamethasone treatment during estrus, is a suppression of luteinizing hormone and possibly ovulation failure (P.M. McCue, personal communication). This side effect has not been observed when dexamethasone is used as a single dose at the time of breeding.

Electro-acupuncture has been used clinically to increase uterine contractility in mares with delayed uterine clearance. Anecdotal reports are encouraging, and research is needed to confirm the efficacy of this treatment alternative.

It is important for the clinician to keep in mind that a transient inflammatory response to semen is normal and required for normal fertility. Post-breeding treatments of these mares will most likely not improve fertility, but may rather cause further contamination and interfere with pregnancy. Only 10-15% of all broodmares develop a pathological persistent form of breeding-induced endometritis.[61] Attention should be given to identify and manage these mares appropriately in order to optimize the reproductive efficiency.

References

1. Pavelko MK, Crabo BG: Possible importance of some sperm coating proteins and their behavior during preservation of boar semen. Proc Int Cong Anim Reprod Artif Insemin 1976. p. 455-462.
2. Crabo BG, Jeyendran RS: Biochemical characteristics of semen and its behaviour during freezing and thawing. Proc Anim Prod Health Div Buffalo Reprod Artif Insem Semin 1979. p. 261-271.
3. Katila T, Sankari S, Makela O: Transport of spermatozoa in the reproductive tracts of mares. J Reprod Fertil Suppl 2000;56:571-578.
4. Troedsson MH, Liu IK, Crabo BG: Sperm transport and survival in the mare: a review. Theriogenology 1998;50:807-818.
5. Gustafsson BK, Graham EF, Crabo BG, et al: Pre-freeze supplementation of ram semen with PGE1 and PGF2 alpha: effects on sperm vitality in vitro and on sperm transport in the ewe. Proc Annu Meet Soc Study Reprod 1977 (abstract).
6. Troedsson MHT, Lee CS, Franklin RD, et al: The role of seminal plasma in post-breeding uterine inflammation. J Reprod Fertil Suppl 2000;56:341-349.
7. Troedsson MHT, Alghamdi AS, Mattisen J: Equine seminal plasma protects fertility of spermatozoa in an inflamed uterine environment. Theriogenology 2002;58:453-456.
8. Troedsson MH, Desvousges A, Alghamdi AS, et al: Components in seminal plasma regulating sperm transport and elimination. Anim Reprod Sci 2005;89:171-186.
9. Rozeboom KJ, Rocha-Chavez G, Troedsson MH: Inhibition of neutrophil chemotaxis by pig seminal plasma in vitro: a potential method for modulating post-breeding inflammation in sows. Reproduction 2001;121:567-572.
10. Alghamdi AS, Foster DN, Troedsson MH: Equine seminal plasma reduces sperm binding to polymorphonuclear neutrophils (PMNs) and improves the fertility of fresh semen inseminated into inflamed uteri. Reproduction 2004;127:593-600.
11. Hunter AG, Nornes HO: Characterization and isolation of a sperm-coating antigen from rabbit seminal plasma with capacity to block fertilization. J Reprod Fertil 1969;20:419-427.
12. Bedford JM: Sperm transport, capacitation and fertilization. In: Balin H, Glasser S, editors. Reproductive biology. Amsterdam: Exerpta Medica; 1972. p. 338-392.
13. Bader H: An investigation of sperm migration into the oviducts of the mare. J Reprod Fertil Suppl 1982;32:59-64.
14. Brinsko SP, Varner DD, Blanchard TL, et al: The effect of postbreeding uterine lavage on pregnancy rate in mares. Theriogenology 1990;33:465-475.
15. Brinsko SP, Varner DD, Blanchard TL: The effect of uterine lavage performed four hours post-insemination on pregnancy rates in mares. Theriogenology 1991;35:1111-1119.
16. Scott MA, Liu IKM, Robertson KR, et al: Acrosomal status and movement characteristics of sperm in the oviducts of normal mares. Proc Int Symp Equine Reprod 1994. p. 173-174.
17. Scott MA, Liu IKM, Overstreet JW: Sperm transport to the oviducts: abnormalities and their clinical implications. Proc Annu Conv Am Assoc Equine Pract 1995. p. 1-2.
18. Bader H, Krause A: Investigations about the transport, distribution and the fate of spermatozoa in the genital tract of the mare. Proc Int Cong Anim Reprod Artif Insemin 1980. p. 197-205.

19. Blair WD, Beck LR: Invivo effects of prostaglandin-F2alpha and E2 on contractility and diameter of rabbit oviduct using intraluminal transducers. Biol Reprod 1977;16:122-127.
20. Rodriguez-Martinez H, Einarsson S: Influence of prostaglandins on the spontaneous motility of pig oviducts. Anim Reprod Sci 1985;8:259-279.
21. Brown SE, Toner JP, Schnorr JA, et al: Vaginal misoprostol enhances intrauterine insemination. Hum Reprod 2001;16:96-101.
22. Woods J, Rigby S, Brinsko S, et al: Effect of intrauterine treatment with prostaglandin E-2 prior to insemination of mares in the uterine horn or body. Theriogenology 2000;53:1827-1836.
23. Brinsko SP, Rigby SL, Lindsey AC, et al: Pregnancy rates in mares following hysteroscopic or transrectally-guided insemination with low sperm numbers at the utero-tubal papilla. Theriogenology 2003;59:1001-1009.
24. Drobnis EZ, Overstreet JW: Natural history of mammalian spermatozoa in the female reproductive tract. Oxf Rev Reprod Biol 1992;14:1-45.
25. Fuchs AR: Uterine activity during and after mating in the rabbit. Fertil Steril 1972;23:915-923.
26. Alexander SL, Irvine CGH, Shand N, et al: Is luteinizing hormone secretion modulated by endogenous oxytocin in the mare? Studies on the role of oxytocin and factors affecting its secretion in estrous mares. Biol Reprod Mono I 1995. p. 361-371.
27. Schams D, Baumann G, Leidl W: Oxytocin determination by radioimmunoassay in cattle .2. Effect of mating and stimulation of the genital-tract in bulls, cows and heifers. Acta Endocrinol (Copenh) 1982;99:218-223.
28. Carmichael MS, Humbert R, Dixen J, et al: Plasma oxytocin increases in the human sexual response. J Clin Endocrinol Metab 1987;64:27-31.
29. Claus R, Schams D: Influence of mating and intra-uterine oestradiol infusion on peripheral oxytocin concentrations in the sow. J Endocrinol 1990;126:361-365.
30. Kendrick KM, Fabre-Nys C, Blache D, et al: The role of oxytocin release in the mediobasal hypothalamus of the sheep in relation to female sexual receptivity. J Neuroendocrinol 1993;5:13-21.
31. Mann T, Lutwak-Mann C: Biochemistry of seminal plasma and male accessory fluids: Application to andrological problems. In: Mann T, editor. Male reproductive function and semen. Berlin: Springer-Verlag; 1981. p. 269-336.
32. Troedsson MH: Uterine clearance and resistance to persistent endometritis in the mare. Theriogenology 1999;52:461-471.
33. Einarsson S, Viring S: Distribution of frozen-thawed spermatozoa in the reproductive tract of gilts at different time intervals after insemination. J Reprod Fertil 1973;32:117-120.
34. Troedsson MHT: Sperm transport and elimination from the mare's reproductive tract. Pferdeheilkunde 2010;26:25-28.
35. Kotilainen T, Huhtinen M, Katila T: Sperm-induced leukocytosis in the equine uterus. Theriogenology 1994;41:629-636.
36. Troedsson MHT, Crabo BG, Ibrahim N, et al: Mating-induced endometritis: mechanisms, clinical importance and consequences. Proc Annu Conv Am Assoc Equine Pract 1995. p. 11-12.
37. Troedsson MHT, Steiger BN, Ibrahim NM, et al: Mechanisms of sperm induced endometritis in the mare. Biol Reprod 1995;52:307 (abstract).
38. Fiala SM, Pimentel CA, Steiger K, et al: Effect of skim milk and seminal plasma uterine infusions in mares. Theriogenology 2002;58:491-494.
39. Troedsson MHT, Desvousges AL, Hansen PJ, et al: Equine seminal plasma proteins protect live spermatozoa from PMN-binding and phagocytosis, while providing a mechanism for selective sperm elimination of apoptotic and dead spermatozoa. Anim Reprod Sci 2006;94:60-61.
40. Troedsson MHT, Doty A, Macpherson ML, et al: CRISP-3 in equine seminal plasma is involved in selective uterine sperm transport. Anim Reprod Sci 2010; In Press.
41. Hughes JP, Loy RG: Investigations on the effect of intrauterine inoculation of *Streptococcus zooepidemicus* in the mare. Proc Annu Conv Am Assoc Equine Pract 1969. p. 289-292.
42. Troedsson MH, Liu IK, Ing M, et al: Multiple site electromyography recordings of uterine activity following an intrauterine bacterial challenge in mares susceptible and resistant to chronic uterine infection. J Reprod Fertil 1993;99:307-313.
43. Alghamdi AS, Foster DN, Carlson CS, et al: Nitric oxide levels and nitric oxide synthase expression in uterine samples from mares susceptible and resistant to persistent breeding-induced endometritis. Am J Reprod Immunol 2005;53:230-237.
44. LeBlanc MM, Johnson RD, Mays MBC, et al: Lymphatic clearance of India ink in reproductively normal mares and mares susceptible to endometritis. Biol Reprod Mono I 1995. p. 501-506.
45. Vanderwall DK, Woods GL: Effect on fertility of uterine lavage performed immediately prior to insemination in mares. J Am Vet Med Assoc 2003;222:1108-1110.
46. Pycock JF: Assessment of oxytocin and intrauterine antibiotics on intrauterine fluid and pregnancy rates in the mare. Proc Annu Conv Am Assoc Equine Pract 1994. p. 19-20.
47. Pycock JF, Newcombe JR: The relationship between intraluminal uterine fluid, endometritis, and pregnancy rate in the mare. Equine Pract 1996;18:19-22.
48. Rasch K, Schoon HA, Sieme H, et al: Histomorphological endometrial status and influence of oxytocin on the uterine drainage and pregnancy rate in mares. Equine Vet J 1996;28:455-460.
49. LeBlanc M, Neuwirth L, Mauragis D, et al: Oxytocin enhances clearance of radiocolloid from the uterine lumen of reproductively normal mares and mares susceptible to endometritis. Equine Vet J 1994;26:279-282.
50. Troedsson MH, Scott MA, Liu IK: Comparative treatment of mares susceptible to chronic uterine infection. Am J Vet Res 1995;56:468-472.
51. Madill S, Troedsson MHT, Santschi EM: Individual registration of longitudinal and circular muscle in the equine endometrium using electromyography and strain gauges: Preliminary data. Havemeyer Foundation International Workshop on Uterine Defense Mechanisms in the Mare: Aspects of Physical Clearance 1997. p. 10 (abstract).
52. Campbell MLH, England GCW: A comparison of the ecbolic efficacy of intravenous and intrauterine oxytocin treatments. Theriogenology 2002;58:473-478.
53. Gunthle LM, McCue PM, Farquhar VJ, et al: Effect of prostaglandin administration post-ovulation on corpus luteum formation in the mare [abstract]. Proc Annu Meet Soc Therio 2000. p. 139.
54. Troedsson MH, Ababneh MM, Ohlgren AF, et al: Effect of periovulatory prostaglandin F2alpha on pregnancy rates and luteal function in the mare. Theriogenology 2001;55:1891-1899.

55. Brendemuehl JP: Effect of oxytocin and cloprostenol on luteal formation, function and pregnancy rates in mares. Theriogenology 2002;58:623-626.
56. Nie GJ, Johnson KE, Wenzel JGW, et al: Effect of periovulatory ecbolics on luteal function and fertility. Theriogenology 2002;58:461-463.
57. Mocklin CM, Paccamonti DL, Eilts BE, et al: Effect of post-ovulatory prostaglandin F2 alpha or cloprostenol on plasma progesterone cncentration in mares. Anim Reprod Sci 2006;94:220-222.
58. Dell'Aqua JA, Jr., Papa FO, Lopes MD, et al: Fertility rates with equine frozen semen after modulation of inflammatory uterine response. Proc Int Cong Anim Reprod 2004. p. 391.
59. Bucca S, Carli A, Buckley T, et al: The use of dexamethasone administered to mares at breeding time in the modulation of persistent mating induced endometritis. Theriogenology 2008; 70:1093-1100.
60. McDonnell AM, Watson ED: The effects of dexamethasone sodium-phosphate on mares with experimentally induced endometritis. J Equine Vet Sci 1993;13:202-206.
61. Zent WW, Troedsson MHT: Postbreeding uterine fluid accumulation in a normal population of Thoroughbred mares: a field study. Proc Annu Conv Am Assoc Equine Pract 1998. p. 64-65.

Management of twins

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Introduction

In spite of improved management techniques, twin pregnancy continues to be a source of economic loss in broodmare production. The introduction of transrectal ultrasound for pregnancy evaluation has revolutionized the identification and management of twin pregnancies in mares. As such, the rate of abortion due to twin pregnancies has dropped from and averge of 20%[1] to 6%.[2] While improvements in management of twins are certainly notable, pregnancy loss as a result of missed twins is costly. Not only does the mare owner suffer lost income from the foal(s), the mare is barren for the season while incurring expenses. As a consequence, management of twin pregnancies to ensure delivery of a viable singleton is essential.

Keywords: Twins, pregnancy, ultrasound

Management of equine twin pregnancies

Ultrasonographic examination of the reproductive tract early in gestation allows for prompt diagnosis and treatment of twin pregnancies.[3] Pregnancies are detected as early as Day 9 of gestation with the aid of transrectal ultrasonography.[4] Diagnosis of twin pregnancies is optimally achieved between Days 13 and 15 of gestation to ensure detection of twins arising from asynchronous ovulations (ovulations occurring more than 24 hours apart).[5]

During the period of embryonic fixation (Day 16-17 of gestation), approximately 70% of twin vesicles will fix in the same horn (unilateral). Of unilaterally fixed twin pregnancies, approximately 85% culminate in natural reduction of one embryo prior to Day 40 of gestation. The remaining embryo develops normally following natural reduction.[6,7]

Bilaterally fixed twin pregnancies (one conceptus in each uterine horn) typically do not result in natural reduction to a singleton conceptus during early pregnancy.[7] Instead, both fetuses often survive until later stages of gestation, at which time abortion usually occurs.[8] Ginther and Griffen[9] recently followed bilateral twin pregnancies in 15 pony mares. In two mares, death of one fetus occurred in the first two months of gestation. Eight of fifteen mares (67%) aborted both fetuses by three months of gestation. Four mares aborted one or both fetuses at 8 months and one mare delivered live twins. The authors noted that early fetal death appeared to be temporally related to the apposition of allantochorions between the fetuses. The actual mechanism causing fetal death is currently unknown.

Management of equine twin pregnancies after 30 days gestation is complicated by the formation of endometrial cups (Day 36 of gestation). The endometrial cup cells secrete equine chorionic gonadotropin (eCG).[10] This hormone is reported to stimulate the formation of secondary corpora lutea thereby leading to increased progesterone production for pregnancy maintenance.[11,12] Endometrial cups remain functional until approximately Day 80-120 of gestation in either the presence or absence of a viable fetus.[13,14] Thompson, et al.[15] demonstrated that irregular estrous cycles and impaired fertility occurred with pregnancy loss in the presence of endometrial cups. Consequently, if both twin pregnancies are lost after Day 35, the mare may not return to fertile estrus for a prolonged time. However, methods for managing equine twins beyond Day 30 of gestation have resulted in inconsistent outcomes. Dietary energy restriction,[16] needle aspiration of one embryonic vesicle per vagina,[17] surgical removal of one vesicle,[18,19] cranio-cervical dislocation,[20] and transvaginal[21-23] and transcutaneous ultrasound-guided[23-25] fetal reduction techniques are methods that have been investigated for twin reduction. This paper will review commonly used techniques to manage twin pregnancies in a clinical setting.

Manual crushing during mobility phase

Although pregnancies can be diagnosed with ultrasound as early as 9 days after ovulation, twin pregnancies are optimally detected between Days 13 and 15 of gestation. During this time period, the embryonic vesicles are mobile within the uterus, and both embryonic vesicles are reliably identified (even after asynchronous ovulation).[4,5,26] Twin pregnancies that are detected during the mobility phase (up to Day 16 or 17) are best managed by manually crushing one embryonic vesicle.[27] Using this procedure, one embryonic vesicle is manually moved to the tip of a uterine horn and crushed under pressure. Survival rates of the remaining vesicle after crushing exceed 90%.[28,29]

The location of the embryonic vesicles is determined with transrectal ultrasound evaluation of the reproductive tract. Early detection of embryonic vesicles may reveal vesicles that are too small to crush. Vesicles

smaller than 10-12 mm are challenging to crush. Further, if the vesicles are located in the uterine body it may be difficult to manipulate them and/or perform an embryonic crush. Re-evaluation the following day(s) may be necessary. Embryos located in different uterine horns are easy to manipulate by milking one vesicle into the tip of a uterine horn for a crush. Embryos that are next to one another or superimposed upon each other may be more challenging to manage. Some recommend re-evaluation of the mare later in the day or the next day to determine if embryo position has changed. When both embryonic vesicles continue to be in close proximity to one another, movement of the vesicle closest to the tip of a horn can be facilitated with manual manipulation. Alternatively, the ultrasound probe can be placed between the vesicles and pressure is used to move one vesicle up the uterine horn for crushing. Using the ultrasound probe to move a vesicle allows for continuous visualization of the vesicle during the procedure. Additionally, the pressure of the probe during movement of the vesicle frequently results in rupture of that vesicle. If the chosen vesicle is still intact once it reaches the tip of the uterine horn, continued pressure can be applied to the vesicle with the fingers or the ultrasound probe. The advantages of performing a crush at the tip of the horn include easy isolation of the vesicle in a smaller diameter uterine lumen and fluid release after rupture that is far removed from the remaining vesicle.

Historically, it is recommended to crush the smaller vesicle or the vesicle that needs the least amount of uterine manipulation.[29] While it is disconcerting to crush the larger of the two vesicles, it might be the wisest choice if the larger vesicle is easily accessed for the operator. Minimizing uterine manipulation is a priority when performing a manual embryonic crush. Further support during the procedure can be provided pharmacologically. To promote uterine or rectal relaxation during the procedure, the mare can be administered N-butylscopolammonium bromide (Buscopan™, Boehringer Ingelheim, St. Joseph, MO; 0.3mg/kg, IV) or propantheline bromide (15mg , IV) can be administered. Treatment during and after the procedure is not consistent between clinicians. Flunixin meglumine is often administered (1 mg/kg, IV) at the time of the procedure to circumvent prostaglandin release during uterine manipulation. Additional administration of flunixin meglumine for the subsequent few days may provide anti-inflammatory support. Exogenous progestins, most frequently altrenogest (Regumate™, Intervet-Schering Plough Animal Health, Millsboro, DE, 0.044 to 0.088 mg/kg, PO, once daily), is administered for enhanced uterine tone and progestin support in the face of luteolysis. Re-evaluation for continued growth and presence of the remaining vesicle should be performed 2-3 days post crushing and every few weeks until placentation occurs.

Manual crush of a twin embryonic vesicle is the preferred method for twin reduction. The procedure is minimally invasive, inexpensive, easy to perform and highly reliable. Additionally, if both pregnancies are lost prior to endometrial cup formation, the mare will cycle back for breeding. The sole reason for not performing a manual crush during the mobility phase would be having missed twins.

Manual reduction after fixation

Manual reduction is preferentially, and most successfully, performed before fixation of the embryonic vesicles. If twin conceptuses are observed after fixation (Day 16-17), manual reduction can be attempted. Manual reduction of unilaterally fixed twins is difficult without damaging both conceptuses. If the vesicles can be separated, 90% of unilateral twins can be manually crushed between days 17-20.[30,31] An attempt at manual reduction of bilateral twins between Days 16 and 40 is a necessity if abortion at a later stage of gestation is to be avoided. Seventy-five percent of bilateral twins may be successfully reduced to a singleton pregnancy by crushing one vesicle before 30 days of gestation. However, with bilateral twins of gestational age >35 days, there is a greater risk of abortion at a later stage if a vesicle is crushed,[30,32] potentially because fluid released from the crushed vesicle gets between the chorioallantois and endometrium and causes a loss of contact.

Transvaginal, ultrasound-guided twin reduction

Selective reduction of pregnancy using transvaginal ultrasonography has been examined in mares having both singleton[33,34] and twin pregnancies.[21-23] The technique involves a 5 or 7.5 MHz transvaginal ultrasound transducer designed for use in large animals. Prior to the procedure, mares are administered broad spectrum antibiotics, flunixin meglumin and altrenogest. Typically, the transducer and casing are cold-disinfected or sterilized prior to placement in the mare. Some individuals cover the transducer with a sterile latex cover (latex ultrasound transducer cover, Civco Medical Instruments, Kalona, IA) or sterile sleeve filled with sterile lubricating jelly. The mare's tail is wrapped and the perineal area cleansed. Ideally, chemical restraint is not use to perform the procedure because of the uterine relaxation induced by some agents (alpha agonists such as xylazine and detomidine hydrochloride). Local anesthetics (2 % lidocaine) can be infused directly into the rectum or mixed with lubricant and carried into the rectum at the time of the procedure. Prior to performing the procedure, the mare often receives flunixin meglumine (1 mg/kg, IV) to counteract prostaglandin release during uterine manipulation. Wearing a

sterile obstetrical sleeve, an operator carries the transducer into the anterior vagina. The operator's arm is then removed from the vagina and placed in the rectum for manipulation of the reproductive tract. The operator manually secures the pregnancy (transrectally) and the transducer is manipulated (transvaginally) until the pregnancy is imaged on the ultrasound screen. The fetal position is clearly identified. A puncture guide on the ultrasound screen is used to select a path for needle placement in the yolk or allantoic sac. An assistant passes a sterile, 16 to 18-gauge, 60 cm needle with an echogenic tip (Echogenic tip spinal needle®, Cook Ob/Gyn, Spencer, IN) through a needle channel in the transducer casing. A sharp jab of the needle is made for passage of the needle through the vaginal and uterine walls into the yolk or allantoic space. After ultrasonographic identification of the echogenic needle tip in the yolk or allantoic space, a 60-ml Luer-tip syringe or suction pump is connected to the needle and fluid is aspirated. To facilitate complete fluid aspiration, the needle can be moved within the sac into areas of detectable fluid. The orientation of the twins (unilateral vs. bilateral) influences when aspiration is discontinued. For unilateral twins, aspiration is discontinued when there is danger of aspirating the fetal membranes between the twins, the conceptus can no longer be visualized because of fluid removal, or it is no longer possible to obtain fluid. When performing the procedure on a bilateral twin, complete fluid evacuation is ideal. Trauma to the treated fetus is not a concern with bilateral twins, and may actually be advantageous.

The success rate of transvaginal ultrasound-guided twin reduction is highly variable and dependent on many factors. In experienced hands, the live foal delivery rate can approach 50% for mares with unilateral twins at Day 35 or less, or mares with bilateral twins up to Day 55 (Jonathan Pycock, personal communication). Day of gestation at the time of reduction appears to impact pregnancy outcome following the procedure. When examining success rates,[21,22,35] there appears to be an advantage to performing the procedure before 36 days of gestation, particularly in the case of unilateral twins. One could argue that unilateral twins prior to Day 40 might reduce naturally and intervention is not necessary.[36] However, by Day 25-30 a size discrepancy is often noted in unilateral twins that are in the process of natural reduction.[7] When twin embryos are similar in size between 25 and 35 days gestation, aggressive management of twins is probably the best option. However, performing the transvaginal ultrasound-guided procedure in mares with unilateral twins has significant limitations due to the close proximity of the embryos/fetuses and associated membranes. One may inadvertently penetrate the adjacent vesicle, and possibly the embryo or fetus, if the placental membranes are not seen in the imaging plane. When aspirating placental fluids for termination of a unilateral twin one can easily aspirate placental membranes into the needle tip causing damage to the remaining fetus. Additionally, when fluid is withdrawn from a unilateral twin vesicle, the adjacent vesicle tends to pull from the endometrium and "fall" into the evacuated space. Fluid may leak from the incompletely evacuated vesicle causing the placental membranes to separate from the endometrium.[34] Direct embryonic/fetal damage, without aspiration, may be advantageous when performing this procedure in unilateral twins. This prevents partial collapse of the vesicle and possible fluid leakage into the endometrium. Treating the mare with exogenous progestins to enhance uterine tone can also help prevent separation of the membranes from the endometrium when performing this procedure. Progestin therapy is generally continued until establishment of placentation (Day 100-120). Mares maintained on exogenous progestins require frequent monitoring to verify fetal viability.

With bilateral twin pregnancies, there is significantly less likelihood that penetration of the conceptus and surrounding membranes will occur. As a consequence, one can more aggressively aspirate fluid from the selected conceptus or induce fetal damage with the needle. Also, the time limitations seen with unilateral twins are not as stringent when using this procedure for bilateral twin preganancies. However, age of the mare, parity, size of the mare, position of the uterus, tone of the uterus can all negatively impact the success of the procedure. The procedure is significantly more difficult if the pregnant uterus is pendulous within the abdomen in aged mares or advanced pregnancy. As a consequence, performing the procedure in older, multiparous mares after 45 or 55 days can be challenging.

Advantages to using a transvaginal ultrasound-guided approach for twin reduction in mares include twin reduction prior to placentation, minimal trauma to the mare and standing, out patient procedure. Disadvantages of this approach include expense, need for specialty equipment and success variability.

Cranio-cervical dislocation

Cranio-cervical dislocation (CCD) is described as the dislocation of the first cervical vertebrae from the cranium, disrupting the ligamentous attachments and severing the spinal cord. This new procedure can be performed to resolve twin pregnancy using transrectal or transabdominal techniques between 60 and 110 days of gestation.[20] The basis for this procedure is to eliminate one twin before placental formation is complete, allowing the remaining fetus to utilize the entire endometrial surface for nutrient and oxygen exchange.

This procedure has been performed using a transrectal approach between 60 and 90 days of gestation. The mare is restrained in stocks or twitched in the doorway. Sedation can be administered as needed, but is generally not

recommended due to uterine relaxation and difficulty in reaching the fetuses. Relaxation of the smooth muscle in the uterus and rectum is generally achieved by the administration of propantheline bromide (15-30mg, IV) or N-butylscopolammonium bromide. These agents facilitate easier identification and manipulation of the fetuses. To help inhibit prostaglandin release, flunixin meglumine (1mg/kg, IV) is administered before the procedure. The smaller fetus or the fetus that has less contact with the endometrium and minimal space to grow is preferentially reduced. This fetus is usually identified in the more cranial aspect of the uterine horn in unilateral twins. Once the targeted fetus is located, the head is isolated by finding the dome-shaped head and palpating the mandible or moving caudally and locating the cervical vertebrae. Cranio-cervical dislocation is performed by stabilizing the head between the thumb and forefinger and bending the head from side to side. This will damage the ligaments attaching the head and neck. Dislocation is then created by placing the thumb at the base of the cranium and applying pressure proximal and dorsally. A distinctive pop is felt if dislocation is achieved, and the thumb and forefinger can be placed in the space created between the head and neck. Mares should be treated with altrenogest at a dose of .088mg/kg once a day once daily for three to four weeks. After cranio-cervical dislocation, death with loss of the fetal heart beat is usually evident within 24 hours to one week. Fetal viability should be evaluated in one week and every two weeks for a month to establish normal growth of the continuing fetus and demise of the other. Using this procedure in eight mares between 55 and 90 days gestation, five (63%) delivered live, singleton foals.

While successful, transrectal cervico-cranial dislocation is technically challenging due to limited proximity of the fetus and potential for rectal injury. A similar procedure for fetal termination has been used via a flank approach (surgical). To date, this procedure has been used for twins between gestational ages of 58 and 150 days. Preoperative medications include: propantheline bromide (30mg IV), flunixin meglumine (1 mg/kg IV), broad spectrum antibiotics (such as penicillin and gentamicin) and exogenous progestins (altrenogest). Propantheline bromide is essential for preventing uterine contractions while finding and manipulating the fetus. Transabdominal ultrasound is used to identify the horn in which the targeted fetus is located. Fetal size and positioning relative to the endometrium are factors considered when selecting a fetus to terminate. A standing flank laparotomy is performed ipsilateral to the horn containing the fetus that has been identified for reduction. Identification of the preferred uterine horn is not always possible because of fetal movements and imaging capabilities. If this occurs, the incision is made in the right flank of the mare, allowing more access to the reproductive tract without intestinal interference. The uterus is located within the abdominal cavity with one arm, and the twin is isolated as described for transrectal dislocations. Cranio-cervical dislocation is performed by manipulating the fetus through the uterus, without incising or invading the uterine lumen. The flank incision is then routinely closed. With this technique, death of the manipulated twin may not be evident until 24 hours to seven weeks. Mares are administered antibiotics and anti-inflammatory agents for 5-7 days and exogenous progestins for at least 30 days after the procedure. Cranio-cervical dislocations, using the surgical procedure, have been performed between 58 and 150days of gestation. Manipulations were only performed once. Cranio-cervical dislocations, using the intra-abdominal surgical procedure, produced a single normal healthy foal in 24 of 38 (63%) of mares. With this technique, death of the manipulated twin was evident from one to eight weeks after the procedure. One fetus that underwent CCD never died and abortion was induced at seven months in order not to affect the mare's present or future fertility. Re-evaluation of fetal viability is performed with transrectal or transabdominal ultrasonography every two weeks until demise of one twin is observed. Signs of impending death of a fetus include: loss of thoracic shape, with the fetus becoming more convex; loss of definition of abdominal organs; and irregular, weak heartbeats.

Delivery of a singleton foals following this procedure is uneventful. Placentas from mares delivering singleton foals have a small sack attached to the allantoic surface. The nonviable fetus is marsupialized into the placenta forming a small pouch, with a stalk protruding from the allantoic surface. This pouch contains the mummified fetal bones. Examination of the chorionic surface reveals minimal evidence that a twin was present, with microvilli present along the entire attachment of the placenta.

Cranio-cervical dislocation has advantages when compared to other procedures for reducing post-fixation twins. The procedure is performed prior to complete placental formation. As such, placental compromise does not occur for the remaining singleton. Anecdotal reports following transcutaneous cardiac puncture for twin reduction suggest that live born singleton foals may be undersized and weak (Johanna Reimer; Lexington, KY, personal communication, April 2001). Additionally, using cervico-cranial dislocation, the uterus is not penetrated with a needle via the abdomen thus reducing the risk of peritonitis and/or fluid leakage which might disrupt the endometrial contact for the remaining twin. Disadvantages of cranio-cervical dislocation include isolation of the fetus and surgical approach. Identifying the correct fetus within the uterus is similar to "bobbing for apples". It is absolutely imperative that the uterus is relaxed enough for identification of fetal anatomy. Additionally, the long period between performing the procedure and fetal death, in some cases, is disconcerting to the mare owner. Little explanation for continued survival of the fetus with a dislocated spinal cord exists.[20]

Transcutaneous, ultrasound-guided twin reduction

The use of transcutaneous ultrasonography to aid in twin reduction in the mare was pioneered by Rantanen and Kincaid in 1988.[24] In experienced hands, an average of 50% of mares undergoing transcutaneous twin reduction will deliver one live foal.[23,24,31] The suggested time to perform this procedure is between 115 and 130 days gestation.[25] As with other described procedures, mares are often treated peri-operatively with broad spectrum antibiotics, anti-inflammatory agents, and exogenous progestins. The procedure is performed in the standing, heavily sedated mare. Sedation promotes movement of the fetuses into the cranial abdomen for easier accessibility and minimizes fetal movement during the procedure. The mare is examined with a 2.5-3.5 MHz transducer to determine fetal position and size. The most accessible fetus is selected for reduction, or when possible, the smaller fetus is targeted. The mare's abdominal area adjacent to the fetuses is clipped and surgically prepared prior to the procedure. The transducer is placed in a sterile obstetrical sleeve containing sterile lubricant. Some veterinarians prefer to infiltrate the area adjacent to the targeted fetus with 2% lidocaine hydrochloride to provide anesthesia prior to passage of the needle. The use of a biopsy guide to perform the procedure is at the discretion of the operator. A biopsy guide on the transducer coordinates with software in the ultrasound which allows the operator to know the expected placement of the needle. Some operators find the use of a biopsy guide limiting when the fetuses and/or mare change position. Typically, a 6 inch, 18-gauge spinal needle is used to perform the procedure. Alternatively, a 6 inch, 18 gauge needle with a stylet and echogenic tip (Cook Veterinary Products, Brisbane, Australia) can be used to allow for better visualization of the needle tip on the ultrasound image. The needle is passed through the skin and abdomen in one motion. Once the needle is passed into the peritoneal space, the needle tip is located on the ultrasound image, advanced through the uterine wall and into the uterine lumen using a quick, thrusting motion. Penetration of the fetal thorax and heart can be challenging as the fetus frequently moves away from the needle if rapid penetration is not achieved. Free flow of blood from the needle after removal of the stylet indicates needle placement within the fetal heart. Potassium chloride (KCl, 2 mEq/ml, up to 32 mEq KCL) or procaine penicillin (10-20 ml)[31] is injected into the fetal heart, thorax or abdomen. Potassium chloride generally results in rapid fetal death, particularly with intracardiac needle placement. Proposed advantages of injecting procaine penicillin for transcutaneous ultrasound-guided twin reduction include: 1) reducing the possible risk of iatrogenic infection, 2) better visualization of the agent as it is injected, and 3) fetal death even in the absence of cardiac placement. A disadvantage of using procaine penicillin to induce fetal death is that it may take up to a few days for the fetus to die (also dependent on injection site).

Cardiac activity of the treated fetus is monitored immediately after the procedure. The fetus does not always die immediately in which case the mare is monitored over subsequent days to assess the status of both the treated and untreated fetuses. Generally, mares are administered flunixin meglumine (1 mg/kg, IV) at the time of the procedure and for up to four additional days (twice daily). Progestin therapy (altrenogest, 0.044 to 0.088 mg/kg, PO, daily) and prophylactic antibiotics are prescribed at the referring veterinarian's discretion.

Success rate of fetal cardiac puncture for twin reduction averages 50% . Factors that contribute to success of the procedure include proximity of the fetus to the adjacent twin (and placentation involved), operator experience and conditions for performing the procedure. As with cranio-cervical dislocation, the terminated fetus is mummified and delivered in a small placental sac along with the live fetus. The terminated fetus rarely interferes with the development of the remaining fetus. However, because the procedure is performed after placental formation is complete, some have speculated that placental insufficiency may result in small, unthrifty foals (Johanna Reimer, personal communication). Other disadvantages of the procedure include potential complications such as peritonitis (rare), need for special equipment and expense.

Elective termination of both pregnancies

When other procedures are not elected or fail to reduce twins to a singleton after endometrial cup formation has occurred, aborting both pregnancies is the last option. Mares carrying twin fetuses have an increased risk for abortion, dystocia, cervical tears and retained fetal membranes. While some mares successfully carry twin pregnancies to term, the risks of complications during pregnancy or delivery warrant termination of one or both fetuses.

Conclusions

Manual reduction of twin embryonic vesicles to a singleton is the most viable option for managing twin pregnancies in mares. In the event that twins are not identified during the period when manual reduction is a

feasible option, several other procedures are available to the mare owner. Pros and cons of all procedures, including success rates and stage of gestation, should be considered prior to selecting a method of management for late embryonic or fetal twins.

References

1. Roberts SJ: Veterinary obstetrics and genital diseases. 3rd ed. Woodstock (vT): Published by the author; 1986.
2. Giles RC Donahue JM, Hong CB, et al: Causes of abortion, stillbirth, and perinatal death in horses - 3,527 cases (1986-1991). J Am Vet Med Assoc 1993;203,1170-1175.
3. ChevalierF, Palmer E: Ultrasonic echography in the mare. J Reprod Fertil Suppl 1982;32:423-430.
4. Ginther OJ: Ultrasonic imaging and reproductive events in the mare. Cross Plains (WI): EquiServices; 1986.
5. Ginther OJ: Twin embryos in mares. I: From ovulation to fixation. Equine Vet J 1989;21:166-170.
6. Ginther OJ: Twin embryos in mares. II: Post fixation embryo reduction. Equine Vet J 1989;21:171-174.
7. Ginther OJ: Postfixation embryo reduction in unilateral and bilateral twins in mares. Theriogenology 1984;22:213-223.
8. Jeffcott LB, Whitwell KE: Twinning as a cause of fetal and neonatal loss in the Thoroughbred mare. J Comp Pathol 1973;83:91-106.
9. Ginther OJ, Griffin PG: Natural outcome and ultrasonic identification of equine fetal twins. Theriogenology 1994;41:1193-1199.
10. Allen W, Moor R: The origin of the equine endometrial cups. J Reprod Fertil 1972;29:313-331.
11. Cole H, Penchars R, GossH: On the biological properties of highly purified gonadotrophin from pregnant mare serum. Endocrinology 1940;27:548-553.
12. Amoroso E, Hancock J, Rowlands I: Ovarian activity in the pregnant mare [letter]. Nature 1948;161:355-356.
13. Clegg M, Boda J, Cole H: Endometrial cups and allantochorionic pouches in mares with emphasis on the source of equine gonadotropin. Endocrinology 1954;54:448-463.
14. Mitchell D: Early fetal death and serum gonadotrophin test for pregnancy in the mare. Can Vet J 1971;12:42-44.
15. Thompson DL, Derrick D, Walker M, et al: Persistent endometrial cups in mares after abortion: effects on the oestrus cycle and fertility. Research Progress Louisiana State University 1982. p. 215-217.
16. Merkt H, Jungnickel S, Klug E: Reduction of early twin pregnancy to single pregnancy in the mare by dietetic means. J Reprod Fertil Suppl 1982;32:451-452.
17. Pascoe RR: Possible new treatment for twin pregnancy in the mare. Equine Vet J 1979;11:64-65.
18. Pascoe DR, Stover SM: Effect of surgical manipulation, placental fluid, and flunixin meglumine on fetal viability and prostaglandin-F2-alpha release in the gravid uterus of mares. Am J Vet Res 1989;50:1505-1511.
19. PascoeDR, Stover SM: Surgical removal of one conceptus from 15 mares with twin concepti. Vet Surg 1989;18:141-145.
20. Wolfsdorf K, Rodgerson DH, Holder R: How to manually reduce twins between 60-120 days gestation using cranio-cervical dislocation. Proc Annu Conv Am Assoc Equine Pract 2005. p. 284-287.
21. Bracher V, Parlevliet JM, Pieterse MC, et al: Transvaginal ultrasound-guided twin reduction in the mare. Vet Rec 1993;133:478-479.
22. Jonker FH, Parlevliet J, Pycock JF, et al: Twin reduction in sixteen mares by transvaginal ultrasound-guided puncture of the embryonic vesicle. Proc Br Equine Vet Assoc Annu Cong 1995.
23. Macpherson ML, Reimer JM: Twin reduction in the mare: current options. Anim Reprod Sci 2000;60:233-244.
24. Rantanen N, Kincaid B: Ultrasound-guided fetal cardiac puncture: a method of twin reduction in the mare. Proc Annu Conv Am Assoc Equine Pract 1988. p. 173-179.
25. Rantanen N: Ultrasound-guided fetal cardiac puncture for twin reduction in mares. Proc Annu Conv Am Assoc Equine Pract 1990. p. 169-171.
26. Palmer E, Driancourt MA: Use of ultrasonic echography in equine gynecology. Theriogenology 1980;13:203-216.
27. Roberts C: Termination of twin gestation by blastocyst crush. J Reprod Fertil Suppl 1982;32:447-449.
28. Pascoe RR: Methods for the treatment of twin pregnancy in the mare. Equine Vet J 1983;15:40-42.
29. Pascoe DR, Pascoe RR, HughesJP, et al: Management of twin conceptuses by manual embryonic reduction - comparison of 2 techniques and 3 hormone treatments. Am J Vet Res 1987;48:1594-1599.
30. Bowman T: Ultrasnoic diagnosis and management of early twins in the mare. Proc Annu Conv Am Assoc Equine Pract 1987. p. 35-43.
31. McKinnon AO, Rantanen N: Equine diagnostic ultrasonography. Baltimore: Williams and Wilkins; 1998.
32. Pascoe RR, Pascoe DR, Wilson MC: Influence of follicular status on twinning rate in mares. J Reprod Fertil Suppl 1987;35:183-189.
33. Squires EL, Tarr SF, Shideler RK, et al: Use of transvaginal ultrasound-guided puncture for elimination of equine pregnancies during days 50 to 65. J Equine Vet Sci 1994;14:203-20
34. Macpherson ML, Homco L, Varner D, et al: Transvaginal ultrasound-guided allantocentesis for pregnancy elimination in the mare. Biol Reprod Mono 1995;1:215-223.
35. Govaere JLJ, Hoogewijs MK, de Schauwer C, et al: Transvaginal ultrasound-guided aspiration of unilateral twin gestation in the mare. Equine Vet J 2008;40:521-522.
36. Ginther OJ: The nature of embryo reduction in mares with twin conceptuses: deprivation hypothesis. Am J Vet Res 1989;50:45-53.

Common causes of abortion

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Abortion, defined as the loss of a pregnancy between 150-300 days, has a severe economic impact on the equine industry. Not only is a mare rendered unproductive for that year, but potentially her reproductive efficiency is decreased the next. The incidence of abortion in mares ranges from five to15% with the upper limit becoming alarming.[1] Older mares that potentially have decreased uterine defense mechanisms and increased fibrosis appear to be at higher risk. Although abortion "storms" are less common than sporadic abortions and noninfectious causes are diagnosed twice as frequently as infectious,[1] when one does occur it is imperative that isolation protocols be implemented immediately. Clinical signs of impending abortion vary considerably from none—in which the mare is found empty during a late-term pregnancy check, or immediately after abortion has occurred—to premature mammary gland development that commonly occurs with placental inflammation and separation. Care must be taken with mares that are being supplemented with exogenous progesterone since death of a fetus can lead to mummification and retention within the uterus rendering the mare infertile. When abortion occurs mares may present empty with the fetal/placental unit intact close by; with the placenta protruding from the vulva lips and a fetus on the ground; or more frequently with just the placenta protruding and no external evidence of a fetus. The last scenario warrants manual uterine exploration for the fetus that usually is present cranial to a non-dilated cervix. Care should be taken delivering the fetus so as to minimize contamination and exposure of humans and mares to potential pathogens. In addition separating the affected mare from the rest of the herd allows decreased potential exposure to infectious agents that have the highest concentrations in placental tissues and fluids. If a mare aborts in a stall appropriate disinfectants should be applied and the mare turned out into an individual pasture, as long as there are no complications (i.e., retained placenta). If a field is contaminated with placental fluids that section should be roped off so mares remaining in that field are not further exposed. These mares should not be moved or mixed with others on the farm until herpes virus, leptospirosis and equine viral arteritis (EVA) testing is negative. Close monitoring of temperatures, serum titers and pregnancy status should be instituted on exposed mares. The greater the distance in time from abortion the less likely an abortion storm agent is responsible. Since 30% of infectious agents are not etiologically identified, submitting both the fetus and complete placenta for gross and histological examination provides the greatest likelihood for a diagnosis.[2] Examination of the placenta on the farm can provide initial information as to whether there was a placentitis, twins, placental edema/anatomical abnormalities, umbilical cord torsion or fetal diarrhea present. In addition uterine culture, leptospira titers and complete physical examination of the mare may provide immediate answers while histopathology is pending.

Keywords: Abortion, equine herpesvirus, equine arteritis virus, leptospirosis, placentitis

The reported causes of abortion and stillbirths in mares change with time, but the differentiation between infectious and noninfectious is still critical in the implications. In a study conducted in central Kentucky during the 1988 and 1989 foaling seasons, placentitis (19.4%) and dystocia-perinatal asphyxia (19.5%) were the two most important causes of equine reproductive loss.[3] Other noninfectious causes of abortion were identified in decreasing frequency as contracted foal syndrome and congenital anomalies (8.5%), twinning (6.1%), improper placental separation (4.7%), torsion of the umbilical cord (4.5%), placental edema (4.3%), bacteremia (3.2%), fetal diarrhea (2.7%) and other placental disorders (6.0%), with a definitive diagnosis not established in 16.9% of the cases.[3] A similar study conducted in the United Kingdom spanning 10 years (1988-1997) concluded that problems associated with the umbilical cord, comprising umbilical torsion (35.7%) and the long cord/cervical pole ischemia disorder (3.1%) were the most common diagnoses of abortion.[3] Twinning (6.0%), intrapartum stillbirth (13.7%) and bacteremia (3.2%) followed as additional non-infectious problems.[4]

Of these noninfectious causes it appears that the ones in which increased knowledge or awareness may have an effect on the outcome includes: twinning, umbilical torsion/pole ischemia and intrapartum/perinatal asphyxia. The use of ultrasonography of the reproductive tract to diagnose pregnancy at an early stage of gestation (11-15 days) has led to a decrease in the incidence of twin abortions. Additionally, most perinatal asphyxias are associated with relative or absolute fetal oversize, fetal malpresentation, maiden mares and unattended foalings. Increasing observation of mares close to foaling and having the necessary support available to correct dystocias and resuscitate the foal can decrease the incidence of loss. Umbilical torsion occurs between six and nine months. The twisted regions result in vascular compromise to the fetus leading to death. The umbilical cords associated with torsion are long; the mean umbilical cord length is 71.97 ± 21.68 cm as compared to 52.36 ± 14.51 cm for cords

associated with normal births.[4] The abnormal length of the cord most often involved is the amniotic portion which shows localized edema, hemorrhagic banding and sometimes urachal dilatation between twists.[3,4] Many foals will remain in utero after death producing autolyzed tissues on presentation. Normal foals have some degree of twisting in utero, but the extent appears to become important when vessels become significantly compressed that the twisting contributes to a lethal outcome. Increased use of fetal monitoring using transabdominal and transrectal ultrasound may have an increased benefit in observing abnormalities or dilatations early. At this time unfortunately there is not much to do unless early termination of the pregnancy is of benefit to the future fertility of the mare.

Infectious agents causing abortion, although overall less common, can be more devastating and produce the most distress among horse owners, managers and veterinarians. Fortunately, improved methods of pre-emptive monitoring, vaccinating, testing, isolating and treating provide a new arsenal with which to combat the diseases. Of infectious agents, bacteria, *Leptospira* spp., a nocardioform actinomycete and *Aspergillus* spp. are most frequently associated with placentitis. Equine herpes virus and EVA should also be considered as important potential pathogens in abortions especially when multiple incidences occur.

Bacterial placentitis is most commonly caused by *Streptococcus* spp. which can be isolated and identified from the placenta and the aborted fetus. Other organisms identified are *Escherichia coli, Pseudomonas* spp., *Klebsiella* spp., and *Staphylococcus* spp.[1,2,5] The most common fungus isolated is A*spergillus*. These organisms gain access to the placenta and therefore the fetus by three characteristic mechanisms. Ascending infection in which the pathogen enters through the cervix causing destruction of microvilli. The lesion appears to spread from the cervical star region moving cranially to the body of the uterus and fetus. Abortion is due to fetal death from septicemia or by the placental insufficiency that occurs with the loss of microvilli. Differentiation between bacterial and fungal lesions is not possible by visual inspection of the placenta. Hematogenous infection occurs when a mare is bacteremic and the organism becomes seeded within the vasculature of the uterus, placenta and fetus. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Staphylococcus, Streptococcus* and *Salmonella abortus equi* are other bacteria that can enter the uterus hematogenously. Luckily all of the above are not contagious and will not usually produce abortion storms except for *Salmonella* (which is transmitted through contamination of pastures with uterine secretions and placental fluids from the mares that aborted). The last means is unidentified and has been attributed to a gram-positive branching bacillus and described as a mucoid placentitis. A nocardioform actinomyte, *Crossiella equi* and *Cullulosimicrobium cellulans* have characteristic lesions of an extensive and severe exudative placentitis centered upon the junction of the placental body and horns rather than the cervical star.[6] Although placental lesions are hard to differentiate visually, *C. cellulans* organisms are absent within chorionic exudates and this bacteria produces prenatal pneumonia and sometimes hepatitis which may be attributed to its motility.[7] In the United Kingdom *Enterobacter agglomerans* has been identified as another organism that can also produce a mucoid placentitis. How these organisms gain access to the uterus and placenta is not known. Clinical signs of placentitis include vaginal discharge and premature lactation; ascending infections are characterized by both and hematogenous and mucoid infections are characterized primarily by premature lactation. In mares that are considered high risk (those that have a history of previous feto-placental compromise, cervical incompetency/lacerations, chronic disease, old age, poor reproductive conformation), monitoring of the uterus and its contents using transrectal and transabdominal ultrasonography and measurement of maternal progestagens and total estrogens, allows early detection of placental and fetal problems (before clinical signs become apparent).[8] These preventative measures in combination enable identification and treatment of a problem early in the course of infection.[9]

For pregnancy to be maintained the fetus needs to develop in a quiet environment, free of infection and inflammation with the placenta providing adequate bloodflow for nutrition and gas exchange. Although bacterial infection initiates disease, based on recent work from an experimental model of ascending placentitis in pony mares, premature delivery may occur secondary to inflammation of the chorion rather than as a consequence of fetal infection.[10] Therefore, therapies are directed at resolving microbial invasion, decreasing inflammation and uterine contractions. Systemic treatment can include antibiotics, exogenous progestagens, anti-inflammatories and tocolytic agents. If a vaginal discharge is present and the cervix open, speculum examination and culture of the exudate provides identification and sensitivity of the organism allowing local treatment and appropriate systemic treatment to be initiated.

Leptospirosis is a zoonotic disease that affects many domestic and wild animals worldwide. Horses are incidental hosts of several leptospiral serovars; only serovar *bratislava* is suspected to be maintained in horses.[11] Serologic surveys demonstrate that leptospiral infection is common in equine populations. However, most leptospiral infections in the horse are subclinical.[12] Clinical disease produces signs that include recurrent uveitis, fever, hemoglobinuria, jaundice, stillbirth and abortion mainly in the last trimester.[11] The most common serovar/serogroup involved in equine abortion is *Leptospira interrogans* serogroup *pomona* serovar *kennewicki*, but rarely, other serovars (*australis*, *grippotyphosa*, *bratislava, icterohaemorrhagica, serjroe*) have also been isolated.[11]

Which serovar presented is usually dependent on the host in a specific area. Leptospiral infection as a cause of equine abortion has been reported as a diagnosis in 3.1% of cases in Hungary, 2.2-3.3% in the USA, in contrast to a 35% prevalence in North Ireland.[11,13] In Kentucky the source of equine infection is thought to come from the wild animal population to include raccoons, skunk, deer, opossum, in addition to cattle and swine. The leptospires are shed in the infected animal's urine contaminating water and feed which are the probable sources of infection for the equine population. Environmental conditions with low-lying swampy areas and stagnant water such as ponds produce higher incidence of disease.

Abortion or stillbirth usually occurs from six months of gestation to term with fetal autolysis present due to death in advance of delivery. The mare usually displays no premonitory clinical signs before delivery, but often has a high antibody titer against one or more leptospiral serovars. The mare is exposed, becomes infected and bacteremic. The organism enters the placenta causing fetal infection with placentitis and funisitis. The gross allantochorion lesions associated with equine leptospirosis consist of nodular cystic allantoic masses, edema, necrotic areas of chorion and necrotic mucoid exudates coating the chorion.[14,15] The microscopic lesions in the allantochorion are thrombosis, vasculitis, mixed inflammatory cell infiltration of the stroma and villi, cystic adenomatous hyperplasia of the allantoic epithelium, villi necrosis and calcification.[2,15]

The gross and microscopic lesions of the umbilical cord include mild to severe edema, focal to multiple sacculations filled with fluid and coating of the surface with a fibrinous exudate, without visible involvement of the three primary blood vessels.[2] A recent report revealed the surface of the umbilical cord diffusely coated by a dense exudates of mostly nondegenerate neutrophils (funisitis) that were mixed with fibrin. These neutrophils only infiltrated the cord surface and minimally into the Wharton's jelly.[15] Gross pathologic lesions of the fetus or stillborn foal include icterus and generalized petechial and ecchymotic hemorrhages. Livers are enlarged, mottled and discolored yellow. Edema is evident in the kidney with pale white radiating streaks in the cortex and medulla.[2,15] Microscopic changes show lesions in the liver and kidney to be the most severe. Liver lesions consist of hepatocellular dissociation, giant cells throughout parenchyma and leukocytic infiltration of the portal triads. The kidneys contain microabscesses with giant cells, dilated tubules, fibrosis and multifocal areas of nonsuppurative interstitial nephritis.[2]

Leptospira sp. can be demonstrated in the allantochorion, umbilical cord, or fetal kidneys by fluorescent antibody tests (FAT), silver staining or immunohistochemistry(IHC).[2,15] Exposure usually occurs two to four weeks before abortion therefore the affected mares have high serological titers. Serology in the mare and fetus is based on ELISA and microscopic agglutination tests. Positive diagnosis in mares occurs with serum titers of $\geq$1:6400.[2] The detection of leptospires by FAT is the method of choice for diagnosing leptospirosis in the kidney of aborted fetuses, although IHC has shown to have a 78% sensitivity and 100% specificity when compared to the gold standard method of culture.[2] Culture however, is not practical since it takes six months for leptospires to grow.

Once an abortion has occurred and leptospirosis is suspected, the mare should be isolated so infective urine and uterine fluids will not expose other mares to the pathogen. Mares that have been pastured with the aborted mare should have leptospira titers drawn to try and identify potentially exposed mares so treatment can be initiated and abortion hopefully prevented. The precise titer level at which exposed mares should be treated is debatable with some feeling it is warranted at titers of 1:100 and others treating at 1:6400. Serial serum titers may better identify exposed mares. Sources of infection such as wildlife (skunks for *kennewicki* and raccoons for *grippotyphosa*), water and contaminated feed should be identified so further exposure does not occur. Treatment is successful with intravenous oxytetracycline (5 mg/kg) once a day or procaine penicillin G (20,000 IU/kg) intramuscularly twice daily for seven to ten days.

Leptospira titers remain high for long periods of time. In addition naturally infected mares shed high numbers of non-host adapted leptospires in urine for up to 14 weeks and therefore mares should not be re-introduced to other mares until shedding has ceased and the mare's urine is negative on fluorescent antibody or silver stain.[2] To collect the urine, furosamide must be administered and then the second void after administration must be collected since more mucus is present initially and may interfere with testing. Dependent on the concentration of the organisms, false negatives may also be a factor (personal communication with Dr. Debra Williams, University of Kentucky Livestock Disease Diagnostic Center; UKLDDC). Further evidence seen by the UKLDDC suggests that a good way to approximate the time when shedding ceases corresponds to decreasing titers (personal communication with Dr. Mike Donahue). This may be a more practical means of determining persistent shedding since the protocol of collecting the urine sample for FA or silver stain is cumbersome and precise.

Equine herpesvirus (EHV) 1 was first isolated in 1932 in Kentucky and has been recognized around the world as a major cause of abortion. However, since the use of preventative vaccination the incidence of loss has decreased over the years. Herpesviridae characteristically infect a susceptible host, replicate and establish a life-long latent infection.[16,17] This cycle of primary infection with periodic episodes of reactivation and shedding of

virus to infect a susceptible host, is the hallmark of herpesviruses and the mechanism by which they persist in the host population.[16,17] Equine herpesvirus1 and EHV4, both alpha herpesviruses, cause respiratory tract infection.[18] However, EHV1 is involved in neurological disorders, equine abortion and neonatal death.

After respiratory infection or reactivation of latent virus, uterine infection occurs via viremia. Transplacental spread of virus then occurs at sites of uterine infarction after endometrial vasculitis and thrombosis, allowing infection of placental trophoblasts, whereby cell to cell spread or infected leucocytes transmit virus to intravillous endothelium and to fetal organs.[19,20] A feature of spontaneous EHV1 abortions in mares is the often sudden and explosive nature of the event, the fetus being expelled without warning and still enveloped within the fetal membranes. Diagnosis routinely includes the detection of EHV1 in the aborted fetus. However some may go undiagnosed because the fetus is not available for examination. Some experimental and field data suggest that the uterus and placenta become more susceptible to EHV1 infection as pregnancy proceeds due to local production of progesterone at the uteroplacental junction, resulting in immunosuppression.[19,20] Transplacental spread of virus occurs at sites of uterine infarction associated with thrombus formation in endometrial arterioles.[19] Virus within the placenta should therefore be concentrated in those areas apposing sites of infarction.

Diagnosis can be made with polymerase chain reaction (PCR) of fresh fetal tissues and paraffin-embedded placenta. Since the virus is transmitted by close contact via aerosol exposure, respiratory secretions, fetal tissues, placenta and uterine fluids from mares that have aborted need to be disposed of and the mare isolated. Virus can be transmitted via organic material on clothes, shoes and material inside stalls, trailers, water buckets or feed. If mares have aborted in the field that area needs to be isolated from the remaining horses in the field, however those horses should not be moved or mixed with other horses. Horses that have been exposed to infected horses but have not developed any clinical signs within 21 days of the potential exposure are unlikely to do so.

Vaccination has decreased the incidence of abortion storms dramatically, with most affected mares exposed coming from naïve herds. Initial recommendations to reduce the risk of abortion from EHV1 and 4 included vaccination with a killed virus vaccine at five, seven, and nine months of gestation. Recently increasing the frequency to every two months year round has been suggested on farms with large movements of mares or that have had endemic problems. Modified live virus vaccines have been used during gestation, however at this time it is an off-label use. Immunity to the virus only lasts four to six months so repeated abortions can occur in successive seasons.

The causative agent of the respiratory and abortagenic disease EVA is a small single-stranded RNA virus (Togavirus).[21] Infection is believed to occur by direct contact via nasal droplet spray during the acute phase of infection and by shedding virus in infected stallions' semen. Susceptible mares that are then bred to shedding stallion acquire the disease. Two carrier states exist in the stallion: a short-term state during convalescence (weeks) and a long term chronic condition which may persist for years.[21] The virus persists in the vas deferens, ampullae, seminal vesicles, prostate and bulbourethral glands and appears to be testosterone dependent. Mares do not appear to become carriers and shedders nor do they pass it via the venereal route. Besides abortion, mares and stallions exposed to the virus do not seem to have permanent or future fertility problems.

Clinical signs range from sub-clinical disease only recognized by seroconversion to acute illness and abortion. Signs are variable and include pyrexia; depression; anorexia; edema of the scrotum, ventral trunk and limbs; conjunctivitis; lacrimation; serous nasal discharge and respiratory distress. Adult horses usually make an uneventful recovery after a viremic phase which can persist for up to 40 days after infection. The incidence of abortion is up to 50% of exposed mares.[21] Abortion may occur with or shortly after infection due to myometrial necrosis and edema leading to placental detachment and fetal death.[21] The causative virus can be isolated from both fetus and placenta, especially from placenta, fetal spleen, lung and kidney and fetal/placental fluids. Semen samples with sperm rich fraction should be collected for virus isolation from suspected infected stallions. Antibodies to EAV can be demonstrated by complement fixation (CF) and virus neutralization tests. The CF test is most useful for studying immunity to arteritis during the first four months after exposure, as the titer peaks two to four weeks after infection and decreases below detectable limits after eight months. Virus neutralization antibody titers develop simultaneously with CF titers, are maximal two to four months after infection and remain stable for several years. Previously infected horses are immune to re-infection with virulent virus for up to seven years. A modified live virus (MLV) vaccine is registered for use in some states in the USA. The use of the MLV vaccine does not produce any side effects apart from a short-term abnormality of sperm morphology and a mild fever with no overt clinical signs. Virus can be sporadically isolated from the nasopharynx and blood for to seven to 32days post-vaccination, so vaccinated horses should be isolated for one month. Horses in contact with and mares served by vaccinated stallions are not infected by EVA. Vaccinated mares bred by positive stallions are protected from clinical infection.

Prevention and control of the disease involves isolation and vaccination of stallions and mares being serviced by infected stallions and maintenance of diligent monitoring of seropositive non-shedding stallions. Stallions shedding the virus should be housed and bred in separate facilities.

Other organisms that have been implicated to a much lesser extent, but should be mentioned, as infectious causes of abortion include; mare reproductive loss syndrome, *Rhodococcus equi, Chlamydophila psittaci, Neospora* sp., *Borrelia parker, B. turicatae* and *Ehrlichia risticii.*

Lastly, another syndrome, fescue toxicosis, should be mentioned since it can have a large impact on a foal crop due to environmental contamination. Tall fescue (*Lolium arundinaceum* [Schreb]) a perennial grass is infected with the endophyte *Neotyphodium coenophialum* which produces toxins that when ingested cause severe adverse effects in late term pregnant mares. Reproductive abnormalities include prolonged gestation, late term abortion, premature placental separation, dystocia, traumatic injury to the reproductive tract, thickened placentas, retained placentas, and agalactia. A high incidence of foal mortality results from prolonged gestation, dystocia with anoxia, dysmaturity, weakness, starvation, failure of passive transfer and septicemia.

Multiple toxins are produced by the endophyte in fescue to include peramines, lolines and ergopeptine alkaloids. Ergot alkaloids act as dopamine D_2 receptor agonists on prolactin secretory cells of the anterior pituitary (lactotrophs) causing decreased prolactin concentrations producing agalactia and decreased priming of the mammary gland for development; decrease tissue binding of estradiol leading to higher serum concentrations of estradiol 17B; inhibit ACTH secretion lowering fetal cortisol concentrations which controls placental function during late gestation. This compromised placental function leads to decreased circulating progesterone and relaxin; lower progesterone levels fail to stimulate lobuloalveolar growth of the mammary gland, which further complicates agalactia associated with increased estradiol-17B and decreased prolactin; may block corticotrophin releasing hormone (CRH) activity in the foal which stimulates adrenocorticotropic hormone (ACTH) release which causes cortisol release from the adrenal. Fetal cortisol increase signals parturition in the mare, therefore lack of fetal production of CRH, ACTH and cortisol could cause prolonged gestation.

Ergovaline and n-acetyl loline have vasoconstrictive properties which may produce hypoxia causing further placental problems. Ergot alkaloids further interact with dopaminergic mechanisms capable of modifying gut motility and affecting the feeding center of the hypothalamus of mature geldings and yearling horses lowering feed intake and digestibility when ingesting endophyte infected hay.

Signs of fescue toxicosis include: prolonged gestation by 20-27 days (360 ± 4 days); lack of signs of imminent foaling (no mammary gland development, hollowing of the paralumbar fossa, softening of the gluteal muscles or relaxation of the tailhead and vulva); dystocia; placental thickening at the cervical star and uterine body and premature placental separation; retained placenta; agalactia or hypogalactia; decreased fertility; weak or stillborn foals (50-86%); dysmature or hypothryroid foals; reduced daily gains of yearlings without supplementation, and increased sweating in pregnant mares.

Prevention and treatment should start 30 days prior to parturition or as soon as possible if knowledge of continued grazing on endophyte infected fescue. Removing the mare from endophyte infected pasture or hay as soon as possible is imperative and attending the parturition is important due to increased risk of dystocia. Drugs used to aid in fescue toxicosis have included:

1) Domperidone at 1.1 mg/kg of body weight/day per os; if administered after foaling domperidone can be given every 12 hours for several days to ensure optimum milk production. As a DA_2 dopamine receptor antagonist it does not cross the blood-brain barrier therefore there is less potential for side-effects. It is effective in resolving pre- and postpartum agalactia and prolonged gestation.

2) Reserpine at 0.01 mg/kg q 24 hours per os. As a Rauwolfian alkaloid it depletes serotonin, dopamine and norepinephrine depots in the brain and other tissues. It is effective in resolving only postpartum agalactia. Unfortunately it crosses the blood brain barrier producing side-effects of sedation and diarrhea; the oral form however has decreased side effects.

3) Sulperide at 3.3 mg/kg/day is a selective DA_2 dopamine receptor antagonist. It has a low prevalence of side-effects even though crosses blood brain barrier. This drug resolves agalactia pre-partum although not as effectively as domperidone.

4) Fluphenizine decanoate at a dose of 25 mg IM one time. As a long-acting D_2-dopamine antagonist it tranquilizes and may predispose animals to extrapyramidal or Parkinson-like side-effects. It is effective in maintaining systemic relaxin and improving pregnancy outcome, however more studies need to be done to determine it value.

References

1. Giles RC, Donahue JM, Hong CB et al : Causes of abortion, stillbirth and perinatal death in horses: 3,527 cases (1986-1991). J Am Vet Med Assoc 1993;203:1170-1175.
2. Donahue JM, Williams NM: Emergent causes of placentitis and abortion. Vet Clin North Am Equine Pract 2000;16:443-456.
3. Hong CB, Donahue JM, Giles RC, et al. Equine abortion and stillbirth in Central Kentucky during 1988 and 1989 foaling season. J Vet Diagn Invest 1993;5:560-566.
4. Smith KC, Blunden AS, Whitwell KE et al: A survey of equine abortion, stillbirth and neonatal death in the UK from 1988 to 1997. Equine Vet J 2003;35:496-501.
5. Macpherson ML, Bailey CS: A clinical approach to managing the mare with placentitis. Theriogenology 2008;70:435-440.
6. Bryant UK: Equine placentitis: common causes and newly emerging pathogens. Equine Dis Q 2008;17:7-8.
7. Bolin DC, Donahue JM, Vickers ML, et al : Equine abortion and premature birth associated with *Cellulosimicrobium cellulans* infection. J Vet Diagn Invest 2004;16:333-336.
8. LeBlanc MM: Identification and treatment of the compromised equine fetus: a clinical perspective. Equine Vet J 1997;Suppl24:100-103.
9. Morris S, Kelleman AA, Stawicki RJ, et al: Transrectal ultrasonography and plasma progestin profiles identifies feto-placental compromise in mares with experimentally induced placentitis. Theriogenology 2007;67:681-691.
10. LeBlanc MM, Giguere S, Brauer K, et al: Premature delivery in ascending placentitis is associated with increased expression of placental cytokines and allantoic fluid prostaglandins E-2 and F-2 alpha. Theriogenology 2002;58:841-844.
11. Szeredi L, Haake DA: Immunohistochemical identification and pathologic findings in natural cases of abortion caused by *Leptospiral* infection. Vet Pathol 2006:43:755-761.
12. Hashimoto VY, Goncalves DD, da Silva FG. Occurrence of antibodies against *Leptospira* spp. in horses of the urban area of Londirna, Parana, Brasil. Rev Inst Med Trop Sao Paulo 2007;49:327-330.
13. Donahue JM, Smith BJ, Poonacha B et al: Prevalence of serovars of *Leptospira* involved in equine abortions in central Kentucky during the 1991-1993 foaling seasons. J Vet Diagn Invest 1995:7:87-91.
14. Bernard W: Leptospirosis. Vet Clin North Am Equine Pract 1993;9:435-443.
15. Sebastian M, Giles R, Robets J et al: Funisitis associated with leptospiral abortion in an equine placenta. Vet Pathol 2005;42:659-662.
16. Gilkerson JR, Love DN, Whalley JM: Epidemiology of equine herpervirus abortion: searching for clues to the future. Aust Vet J 1998;76:675-676.
17. Ostlund EN: The equine herpesviruses. Vet Clin North Am Equine Pract 1993;9:283-294.
18. Reed SM, Toribio RE: Equine herpesvirus 1 and 4. Vet Clin North Am Equine Pract 2004;20:631-642.
19. Gerst S, Borchers K, Gower SM, et al: Detection of EHV-1 and EHV-4 in placental sections of naturally occurring EHV-1 and EHV-4 related abortions in the UK: use of the placenta in diagnosis. Equine Vet J 2003;35:430-433.
20. Smith KC, Whitwell KE, Blunden AS et al: Equine herpesvirus-1 abortion: atypical cases with lesions largely or wholly restricted to the placenta. Equine Vet J 2004;36:79-82.
21. Chirnside ED: Equine arteritis virus: an overview. Br Vet J 1992;148:181-193.

Dystocia management in equine practice

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Abstract

Equine dystocia is a true emergency for the equine practitioner. After stage two labor begins, there is a limited amount of time to correct a maldisposition and deliver a viable foal. The equine practitioner needs to be prepared to efficiently evaluate the condition of the mare and fetus, and to determine the safest and most effective method of resolving the difficult delivery. Then, the practitioner must be prepared with proper equipment, supplies, and personnel in order to intervene quickly and effectively so that a successful outcome can be obtained. In most cases, mutation with assisted vaginal delivery is sufficient for resolution and delivery of a viable foal. However, for a prolonged unattended dystocia with resultant trauma to the mare's genital tract, severe maldisposition of the fetus, or certain fetal malformations, the mare may need to be taken to a referral hospital for a controlled vaginal delivery, fetotomy, or cesarean section. There are three key factors that determine the success of the equine practitioner's dystocia management: 1) the condition of the mare and fetus upon presentation, 2) the expedient and skilled response by the practitioner, and 3) the equipment and referral services that are readily available to the practitioner and client. Additionally, post-dystocia care of the mare and foal is critical to a successful outcome for both the mare and foal.

Keywords: Dystocia, equine, mutation, controlled vaginal delivery, maldisposition, parturition

Introduction

Equine dystocia, or abnormal or difficult foaling, is a true emergency in equine practice. Dystocia occurs in less than 1% of equine parturitions.[1] Mares have fewer problems with deliveries than any other domestic species; however, when difficulties are present, they are true emergencies because of the rapid and strenuous nature of the delivery process. The incidence of dystocia varies according to breed. For example, studies have found the incidence of dystocia to be around 4% in Thoroughbreds, 10% in Belgian Draft horses, and 8% in Shetland Ponies.[2] Generally, there is a higher incidence of dystocia in these breeds because the Thoroughbred foal has relatively long fetal extremities, the Belgian foal is prone to fetal muscular hypertrophy, and the Shetland foal can have an oversized skull, approaching hydrocephaly in some cases.

The time from when stage two of parturition begins (rupture of the chorioallantois) until the foal has been delivered is critical in determining the survival and health of the foal in the neonatal period and the future reproductive health of the mare. The normal duration of stage two labor is 20–30 min from the time the chorioallantoic membrane ruptures to delivery of the foal.[3] Foal survival rates are low if the foal is not delivered within 40 minutes from the onset of stage two labor.[4] There have been live foals delivered up to 90 min or longer after the onset of stage two labor, but these cases are the exception to the rule.

The most common problem seen as a result of a prolonged delivery is hypoxia in the foal. This can be caused by premature placental separation, pinching off of the umbilical cord from prolonged time spent in the birth canal, and/or inability to expand the foal's thoracic cavity because of prolonged external pressure from the mare's bony pelvis. The strong abdominal press of the mare and her unpredictable behavior during stage two labor make it difficult and often dangerous to intervene unless the practitioner is experienced and skilled. The practitioner must be prepared to act swiftly and effectively to minimize fetal hypoxia, as well as prevent excessive trauma to the mare's reproductive tract.

The condition of the soft tissues of the birth canal is critical in determining the future fertility of the mare. For this reason, repeated insertion and removal of the clinician's hand and arm can easily abrade the vaginal and vestibular tissue of the mare. When these mucous membranes are abraded, the mare is prone to post-procedure systemic infections, endotoxemia, laminitis, and fibrosis and adhesions of the cervix, vagina, and vestibule after healing. In order to act expeditiously, the practitioner must have the proper equipment and supplies readily available (Table 1).

Table 1. Equipment suggested for a dystocia in the field.

OBSTETRICAL LUBRICANT
Sterile Stomach Pump and Stomach Tube
Obstetrical Chains and Handles
Head Snare

It is important that the clinician understand the normal presentation, position, and posture of the fetus at delivery. Normal delivery is defined as a cranial longitudinal presentation, with dorsosacral position and the fetal head, neck, and forelimbs extended. Maldisposition of the foal refers to an incorrect presentation, position, and/or posture of the fetus at the time of labor. Fetal presentation describes the orientation of the fetal spinal axis to that of the mare's spinal axis and also the portion of the fetus that enters the vaginal canal first. A cranial longitudinal presentation is normal, where the fetal head is presented first at the vulva. Position is defined as the relationship of the dorsum of the fetus to the quadrant of the maternal pelvis; dorsosacral positioning is normal. Posture refers to the relationship of the fetal extremities to the fetal trunk. The clinician's ability to quickly and accurately diagnose and determine the presentation, position, and posture of the foal during a dystocia is critical to a successful outcome.

Fetal causes of dystocia account for the majority of difficult deliveries. The primary fetal cause of equine dystocia is postural abnormalities of the long fetal extremities. Large equine fetuses are less likely to cause dystocias than large bovine fetuses because of the round shape of the mare's pelvis as compared to a cow's pelvis. Other less frequently encountered fetal causes of dystocia include fetal anasarca, fetal ascites, fetal tumors, hydrocephalic fetus, fetal monsters and mummified fetuses. In a 1997 study of 150 obstetrical cases from two equine referral hospitals, it was apparent that severe dystocia is often multifactorial, with 86% of the cases involving malposture and 58% of these involving more than one extremity. In 30% of the cases, malposition was a factor, and abnormal presentation was involved in 24% of the cases.[5]

Maternal causes of dystocia include uterine torsion, abnormal size and/or shape of the pelvis, uterine inertia, immaturity, constriction of the cervix or vagina, ruptured prepubic tendon and abnormal abdominal musculature. Primiperous mares account for approximately 30% of referral hospital dystocia cases, a disproportionate number considering that many present solely because it is their first foal, with no apparent maldisposition of the foal.[2,6-8]

Diagnosis

When a client calls with an apparent dystocia, the practitioner's initial instruction should be to keep the mare up and walking until the clinician arrives at the farm. This helps prevent the mare from injuring her reproductive tract with continued excessive and nonproductive straining with a maldispositioned foal. Upon arrival at the farm, a quick history should be taken: due date, elapsed time since stage two of parturition began, previous foaling difficulties, previous health issues of the mare during pregnancy, and any history of pelvic fractures. A quick examination of the general condition of the mare should also be performed, including heart rate, mucous membrane color, and overall condition. The clinician should note the mare's behavior, posture, respiration, ability to stand and walk, and the presence of the amnion and fetal extremities protruding from the vulva. Finally, a vaginal examination should be performed to assess the posture, presentation and position of the foal. The reproductive tract and fetus should be examined for abnormalities such as visceral organs in the vagina, fetal viability, and caudal reproductive tract lacerations. Genital examination and obstetrical procedures should be performed as sanitarily as possible.

Several factors should be considered in determining if the dystocia can be managed at the farm, including the economic value of the fetus and dam; time since onset of stage two labor; distance to a hospital; clinical skill and preparedness of the clinician; amount of assistance available at the farm; presentation, positioning and posture (disposition) of the fetus on examination; and the systemic and genital tract health of the mare upon initial evaluation.

Treatment

There are several commonly employed methods for correction of dystocia. These include mutation with assisted vaginal delivery, controlled vaginal delivery, fetotomy, and cesarean section. Fetotomy, controlled vaginal delivery, and cesarean section are better handled in a hospital setting. Although a fetotomy can be performed in a standing mare under epidural anesthesia, the author prefers to perform fetotomies with the mare under general anesthesia in dorsal recumbency. Additionally, a fetotomy requires at least one, and preferably two competent

assistants, which is better accommodated at a hospital. Controlled vaginal delivery, by definition, has the mare under general anesthesia in dorsal recumbency with her hind limbs elevated to help promote repulsion of the fetus back into the uterus and cranial to the mare's pelvic inlet; this is most easily accomplished in a hospital setting. Additionally, with an anesthetized mare, the foal will also be anesthetized to a certain extent; therefore, a foal resuscitation team with appropriate medications and equipment should be standing by awaiting the delivery of a potentially viable foal. Cesarean section, unless it is going to be a terminal procedure, needs to be performed in a controlled environment where sterility can be maintained. No matter where a dystocia is managed, care should be taken to monitor the time that has elapsed so that decisions can be made to abandon one technique for another and the dystocia resolved in a timely fashion.

Sedation

Chemical sedation should be used with discretion to prevent cardiovascular and respiratory compromise of the mare and fetus. The author prefers to work at the farm without sedation; the inability to properly resuscitate the foal supersedes the benefits of sedation in this location. The sedatives commonly used in equine practice (xylazine hydrochloride [TranquiVed, Vedco Inc., St. Joseph, MO], detomidine hydrochloride [Dormosedan®, Pfizer Animal Health, Exton, PA], acepromazine maleate [Acepromazine Maleate Injection, Phoenix Pharmaceuticals Inc., St. Joseph, MO], and butorphanol tartrate [ButorJect®, Phoenix Pharmaceuticals Inc., St. Joseph, MO]) are not approved for use in the pregnant mare. They should be used judiciously to prevent compromise of the cardiovascular and respiratory systems of the mare and fetus. Acepromazine has little effect on the fetus and is generally considered safe for use in the pregnant mare; however, myometrial activity was reported to decrease following administration of acepromazine to normally cycling mares. Detomidine causes significant fetal and maternal cardiovascular compromise and likely diminished placental perfusion. The fetal depressant effects of xylazine are of shorter duration than are those of detomidine. Detomidine and xylazine cause some mares to become hypersensitive over their hindquarters despite appearing to be well sedated. Myometrial activity increases after administration of detomidine or xylazine in mares.[9] Although these drugs may be useful in calming the mare and keeping the obstetrician and personnel safe, these side-effects should not be ignored.

Mutation

Mutation with assisted vaginal delivery is the most frequently used method for resolving a dystocia. Most dystocias can be successfully managed at the farm fairly quickly via this method. Mutation is the procedure used to correct maldisposition, followed by delivery of the entire fetus. The practitioner manipulates the foal by repulsion, rotation, version, and/or adjustment or extension of the fetal extremities to enable vaginal delivery. The practitioner should make an attempt to avoid unnecessary abrasion of the vaginal mucosa by limiting the number of times his or her arm is inserted and removed into the mare's vagina and by using plenty of lubricant.

Mutation with assisted vaginal delivery is indicated when the clinician feels that there is adequate room within the uterus/pelvic area and there is minimal adjustment required. Additionally, the duration of the dystocia should be taken into consideration. As more time elapses, there will be less room in the pelvic area, which may necessitate repulsion of the fetus into the abdominal cavity to create more space for manual correction of deviated limbs and/or the use of lubricants to assist in manipulation. Copious amounts of lubrication should be the practitioner's first line of treatment for most dystocias. If the foal has not been delivered after 15 min of attempting mutation, other options need to be considered.

There are several scenarios where the mare should be referred directly to the referral hospital. This is generally indicated if it will take the practitioner more than 15 min to get to the mare's location, if the foaling attendant is very experienced and informs the practitioner that there is a severe maldisposition, or if the mare and/or fetus are very valuable.

Controlled vaginal delivery

Once the decision has been made to transport the mare to a hospital, the dystocia team is quickly assembled: anesthetist, obstetrician, and neonatologist, along with the appropriate technical assistance and the necessary equipment (Table 2).

Table 2. Equipment necessary for a controlled vaginal delivery in the hospital.

INDUCTION/RECOVERY STALL WITH ANESTHESIA EQUIPMENT AND HOIST
Sterile Stomach Pump and Stomach Tube
Obstetrical Lubricant
Obstetrical Chains and Handles
Head Snare
Nasotracheal Tube
Ambu Bag
Oxygen Supply
Emergency Drugs and Supplies for Neonatal Resuscitation

When the mare arrives in the hospital, a brief assessment of maternal and fetal condition is performed to determine if the mare is able to withstand general anesthesia. If the mare is systemically ill or is believed to be a poor candidate for general anesthesia, then she will be considered a candidate for a standing fetotomy. If she can withstand general anesthesia, she is taken to the induction stall and anesthetized. This evaluation and induction of anesthesia should not take more than five minutes.

Once recumbent, hobbles are placed on all four pasterns and hooked onto a hoist. The mare is lifted and appropriate padding is placed under her. She is lowered, and the front hobbles are removed from the hoist. The hindquarters are then lifted again until the pelvis is approximately three feet above the floor. At this time the mare is placed on gas anesthesia, and fluids are given to assist with recovery (5 L of lactated ringer's [LRS; Lactated Ringer's, Baxter Healthcare Corporation, Deerfield, IL] with 500 mL of calcium gluconate [Calcium Gluconate 23% Solution, Vedco Inc., St. Joseph, MO]). The disposition of the fetus is examined, and copious amounts of general obstetrical lubricant (Equi-Phar Vedlube, Vedco Inc., St. Joseph, MO) are pumped into the uterus via stomach tube and stomach pump. The relaxed uterus, the copious amount of lubricant, and the effects of gravity all assist the obstetrician in repulsion and repositioning of the fetus. After the foal is in normal presentation, posture, and position, obstetrical chains are placed on both forelimbs, the mare is lowered to lateral recumbency, and handles are placed on the obstetrical chains. The fetus can then be extracted via traction by a controlled vaginal delivery. Following delivery of a live foal, the umbilical cord is clamped and transected, and the foal is placed on a gurney so the head can be lowered to drain fluid out of the nasal passages, trachea, and lungs. A nasotracheal tube is passed, if necessary, to ensure a patent airway, and oxygen and an ambu bag are available, if needed, as are emergency drugs, intravenous catheters, and intravenous fluids.

After extraction, the mare's hindquarters are raised, and a large volume of dilute povidone iodine solution (Prodine Solution, Phoenix Pharmaceuticals, Inc., St. Joseph, MO) is pumped into the uterus to assist with removal of the lubricant. The mare is then lowered to allow the fluid to drain from the uterus. She is raised again, the excess fluid and lubricants are removed from the exteriorized parts of the placenta, and these parts are placed back into the uterus. The placenta should not be pulled out, as the relaxed uterus will also likely come out with the placenta at this time. Surgical towels are placed within the vestibule, and towel clamps are placed on the vulva to hold the towels and placenta inside the mare. Water is used to rinse the lubricants from the mare's hindquarters, and the floor is cleaned underneath the mare. She is lowered again, and the hobbled front legs are placed back on the hoist. The mare is then lifted onto a surgical table and moved to a clean recovery stall. If a second recovery stall is not available, care must be taken to clean the floor sufficiently before the mare awakes to ensure that the surface is not slick from the fluids and lubricant used/expelled during the procedure. After recovery, the towel clamps and towels are removed, and the placenta is allowed to hang out of the mare as it would after a normal delivery.

Fetotomy

Fetotomy should only be considered when proper restraint of the mare is obtainable, when the obstetrician has experience performing fetotomies, and when proper fetotomy equipment is available (Table 3).

Table 3. Equipment necessary to perform a fetotomy.

FETOTOME (UTRECHT MODEL)
Wire Threader
Wire Saw Handles
Wire and Wire Cutters
Wire Introducer
Krey Hook
Fetotomy Knife
Obstetrical Lubricant
Sterile Stomach Pump and Stomach Tube
Obstetrical Chains and Handles
Head Snare

If a fetotomy is attempted without all of these critical factors, extensive damage can occur to the mare, which can limit or eliminate her reproductive future, or, in some cases, maternal death may occur. If the mare already has extensive damage to her caudal reproductive tract, a cesarean section should be performed to avoid further damage. When the foal is already dead, it is preferable to use a fetotomy; it may be simpler and less damaging to the mare's reproductive tract to make one or two simple cuts instead of working at the more time consuming mutation that may not resolve the problem and will still cause some damage to the reproductive tract. Fetotomy should also be considered when there are certain types of maldispositions, such as dog sitting (when head and forelimbs are outside of the mare with one or both hind limbs flexed at the hip), wry neck (when the neck is twisted back in a U-shape and cannot be straightened), or severely contracted tendons of the fetal carpus and/or elbow.

The author prefers to perform fetotomies in a hospital setting under general anesthesia. However, fetotomy in the standing position is possible under sedation with epidural anesthesia. In this case, an epidural with .75 mL xylazine and 2.5 mL of 2% lidocaine hydrochloride (Lidocaine Hydrochloride Injectable-2%, Vedco, St. Joseph, MO), qs up to 6 mL with sterile saline, is administered via a 3.5-in, 20-ga. spinal needle.

Cesarean section

After initial assessment of the fetal position and mare's reproductive tract, if the foal is still alive and the cause of the dystocia cannot be corrected quickly (15–20 min), or if it is determined that extensive manipulation is required, a cesarean section should be performed. Indications for a planned cesarean section include a previously fractured maternal pelvis or any other obstruction that reduces pelvic diameter (e.g., tumors in pelvic canal), abdominal wall or prepubic tendon ruptures, and uterine torsions in late gestation that cannot be corrected by other means. A cesarean section may also be indicated when excessive trauma to the mare's caudal reproductive tract is diagnosed on initial assessment. As with the controlled vaginal delivery, a neonatal team should be ready to resuscitate the foal after it is delivered. Obviously, a caesarean section where the mare will be saved needs to be performed in a surgical environment where sterility can be maintained. Cesarean section is most advantageous when it is preplanned and the time and facilities are immediately available.

Post-dystocia care for controlled vaginal delivery, fetotomy, and cesarean section

The mare is moved to a stall after she recovers from anesthesia (a neonatal stall if the foal survived or a standard stall if the foal died) or after the fetotomy has been finished. Mares that have had obstetrical manipulations are predisposed to retention of fetal membranes, so proactive treatment for metritis, laminitis, and endotoxemia is warranted.[10] At this time she is given a 10 L bolus of LRS and is started on routine therapy of systemic antibiotics (typically penicillin G potassium [Pfizerpen®, Pfizer Inc., New York, NY] and gentamycin sulfate [GentaMax® 100, Phoenix Pharmaceutical, Inc., St. Joseph, MO]), non-steroidal anti-inflammatory drugs (flunixin meglumine [FluMeglumine®, Phoenix Pharmaceutical, Inc., St. Joseph, MO]), and anti-ulcer medications (omeprazole [Gastroguard®, Merial Limited, Duluth, GA]). Ice boots and pentoxifylline (Pentoxifylline Extended-Release Tablets, Apotex Corp, Weston, FL) are used to deter laminitis. An abdomenocentesis and a CBC are performed 12-24 hours after dystocia (controlled vaginal delivery or fetotomy) to determine if there was any abdominal contamination owing to a uterine laceration. The birth canal should also be evaluated and palpated for evidence of

lacerations. Assuming that the analysis of the abdominal fluid is normal and/or there is not a full thickness laceration, a large volume uterine lavage is performed at least once, and sometimes twice, daily (~5 gal dilute povidone iodine), along with administration of oxytocin (Oxytocin Injection, Osborn®, Le Sueur, MN) and an intrauterine infusion of oxytetracycline (Oxymycin 200®, Agripharm Products, Westlake, TX; 5 g qs to 60 mL with sterile saline).

Conclusion

Equine dystocia is a true emergency for the equine practitioner. The objective of dystocia management, whenever possible, is to save the life of both the dam and the fetus. After stage two labor begins, there is a limited amount of time to correct a maldisposition and deliver a viable foal . The equine practitioner needs to be prepared to efficiently and effectively evaluate the condition of the mare and foal. Then, the practitioner must be prepared with proper equipment, supplies, and personnel in order to intervene quickly and effectively so that a successful outcome can be obtained. In most cases, mutation with assisted vaginal delivery is sufficient for resolution and delivery of a viable foal. However, for a prolonged unattended dystocia with resultant trauma to the mare's genital tract, severe maldisposition of the fetus, or certain fetal malformations, the mare may need to be taken to a referral hospital for a controlled vaginal delivery, fetotomy, or cesarean section. There are three key factors that determine the success of the equine practitioner's dystocia management: 1) the condition of the mare and fetus upon presentation, 2) the expedient and skilled response by the practitioner, and 3) the equipment and referral services that are readily available to the practitioner. Additionally, post-dystocia care of the mare and foal is critical to success. There are two very different patients with very different needs to address. Attention to details and expertise are crucial to a successful outcome for both mare and foal.

References

1. Threlfall WR: Parturition and dystocia. In: Youngquist, RS, editor. Current therapy in large animal theriogenology. Philadelphia: Saunders; 1997. p. 141-153.
2. Vandeplassche M: Dystocia. In: McKinnon AO, Voss JL, editors. Equine reproduction. Philadelphia: Lea and Febiger; 1993. p. 578-595.
3. Youngquist RS: Equine obstetrics. In: Morrow D, editor. Current therapy in theriogenology. 2nd ed, Philadelphia: Saunders; 1986. p. 693-699.
4. Roberts SJ: Veterinary obstetrics and genital diseases (theriogenology). 3rd ed. Woodstock (VT): Published by the author; 1986. p. 287-352.
5. Fraser, G, Perkins N, Blanchard T, et al: Prevalence of fetal maldispositions in equine referral hospital dystocias. Equine Vet J 1997;29:111-116.
6. Giles RC, Donahue JM, Hong CB, et al: Causes of abortion, still-birth, and perinatal death in horses: 3527 cases (1986-1991). J Am Vet Med Assoc 1993;203:1170-1175.
7. Jean D, Laverty S, Halley J, et al: Thoracic trauma in newborn foals. Equine Vet J 1999;31: 149-152.
8. Vandeplassche M: Selected topics in equine obstetrics. Proc Annu Conv Am Assoc Equine Pract 1992; p. 623-628.
9. LeBlanc MM, Norman WM: Sedatives and anesthesia of the mare during obstetric manipulation. Proc Annu Conv Am Assoc Equine Pract, 1992; p. 619.
10. Vandeplassche M: Aetiology, pathogenesis and treatment of retained placenta in the mare. Equine Vet J, 1971;3:144.

Induced lactation nurse mares used to raise Standardbred sale yearlings

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Abstract

The use of nurse mares is common in the horse breeding industry for reasons ranging from orphan foals to management of difficult mares. An alternative to parturient nurse mares is the induction of lactation in barren mares. Mares selected must have raised a foal previously and exhibit good maternal behavior. Over two years at a Standardbred breeding farm in central Kentucky, 35 mares from a herd of retired or adopted broodmares raised sale yearlings. All mares given a foal adopted it completely and raised it to weaning without incident. In the first year a combination of domperidone, progesterone and estrogen was used to induce lactation. In the second year domperidone alone was used after exposure to artificial photoperiod. Better lactation results were obtained in the first year. Sale results from the first crop of foals raised showed no drop in sale price as a result of being raised by induced lactation mares. This indicates that these mares raise a foal indistinguishable from a naturally raised foal.

Keywords: Induced lactation, nurse mares, foal adoption

Introduction

Nurse mares are commonly employed to raise orphan foals, foals from poor dams, foals from difficult to breed dams or foals whose dam must be shipped for breeding. These nurse mares are traditionally mares which have recently foaled, meaning their own foal is replaced with the adopted foal. The generation of these "nurse mare foals" has become an animal welfare concern which has stigmatized the use of purpose-bred nurse mares. Another negative to the use of these nurse mares in large breeding operations is the introduction of diseases into the herd and facilities when the nurse mares are employed. The fact that nurse mares are sent to farms to raise foals, return to a central location to foal and then disseminate the following year to new farms means they may easily spread communicable diseases from on farm to another.

An alternative to a parturient nurse mare is to induce lactation in a mare. Previous reports have shown success generating an adequate milk supply in non-parturient mares provided they have raised a foal previously. Reports of successful adoptions and raising of foals has been presented previously,[1-3] but the adoption protocols have been time-consuming.[2] For large-scale commercial use of these mares the adoption protocol must be quick, reliable and repeatable and the mares must be able to successfully raise foals indistinguishable from foals raised on parturient nurse mares on their natural dams.

Due to semen shipping restrictions imposed by some state breed incentive programs, some mares must be bred in certain states. Rather than ship or foal those mares off-farm, our farm has made frequent use of nurse mares to raise the foals from dams which must be shipped cross-country for breeding in other states. This allows us to foal on the farm and raise these foals while shipping the mares to the states required for breeding. The frequent use of nurse mares on this farm has resulted in increased disease levels within the foal population, increased cost associated with raising foals and increased labor needs for breeding the nurse mares. Non-parturient induced lactation nurse mares were investigated in an effort to control the traffic of horses on the farm, establish disease control, decrease costs and eliminate production of unwanted foals.

Mare selection

Mares to be used for induction of lactation were adopted from rescue organizations or recruited from our retired mare herd. The year one herd reached a maximum of 24 mares, of which induced lactation was attempted on 23. The herd consisted of 13 Standardbreds, eight Quarter Horses and three Thoroughbreds ranging in age from seven to 22 years old. One Quarter Horse mare was added in the second year. Twenty-three of the 25 mares had known reproductive histories and raised a foal previously. Foaling had occurred between one and five years previously in these mares, with the mares having raised between one and 12 foals. To be selected a mare had to be in good physical condition, easy to handle for injections or oral medications, willing to have her mammary gland manipulated and have no history of prior negative maternal indicators. Mares were held for at least one month on the farm before attempting to induce lactation.

Induction of lactation

Year 1: Based on previous studies[1,3] and discussions with local practitioners, a sulpiride based protocol was attempted at first in year one of the study. This protocol did not result in significant milk production in our mares and was abandoned after the second mare. Both mares were switched to a domperidone based protocol as

were the remainder of mares in this study. Twice daily oral domperidone at twice the normal dose (2.2 mg/kg PO) was administered. Mares were given IM progesterone and estrogen (150 mg and 10 mg, respectively) once daily. Mares were not milked until adoption. Mares were observed daily for milk production and filling of the teats. Mares remained on the domperidone and steroids until adoption was attempted, ranging from six to16 days. All 22 mares received this treatment with the earliest beginning on February 6 and the latest beginning on May 4.

Year 2: All mares were placed under lights to maintain a photoperiod of 16 hours daily starting on December first. Domperidone (2.2 mg/kg PO twice daily) was used without the addition of exogenous steroids. Seventeen mares were used this year, with 16 being mares used the previous year and one mare added to the herd in the middle of the season. Mares were selected based on ease of handling and successful milk production the year before.

Adoption protocol

Mares were considered ready to adopt a foal when they had "filled" within one hour from being manually milked. The dam of the foal to be adopted was moved to a separate barn and the foal left alone for one hour prior to adoption. Foals were between 16 hours and 13 days old at adoption. The nurse mare was placed into stocks with an adjacent small foal stall approximately eight feet by eight feet. The mare stocks have solid walls with a padded hole large enough for the foal to nurse (Figure 1). The rear of the stocks is the height of a normal palpation stock and allows for cleaning and manipulation of the mare. The mare was held with a lead rope or chain over her nose depending on disposition. The mare was given dinoprost (5 mg, IM), oxytocin (5 IU, IV), and romifidine (20 µg/kg IV). Immediately after the injections the mare's tail was tied with brown gauze and the mare was cleaned as for breeding. By this time the mare was usually sedated and starting to sweat and cramp from the dinoprost. The foal was brought into the stall and loosely held with the foal's hindquarters near the mare's head. The foal was not allowed to assume a nursing position at this time. Vaginal and cervical massage was begun and continued for two to four minutes. The cervix was dilated after initial massage and the interior of the cervix was gently massaged. During this time most mares would nicker or lick the foal and begin to show interest in it. After the cervical stimulation the foal was allowed to assume a nursing position and attempt to nurse. The mare was corrected by voice cues or a quick response on the lead if she acted aggressively to the foal at this time. Mares which would not allow the foal to nurse were restrained only if they were a risk to the foal. After nursing, the foal was removed from the stall. If the mare responded by vocalizing and becoming anxious in the stocks they were both taken to a stall and the mare held on the lead rope while her behavior towards the foal was monitored. The mare was released when the foal could approach and nurse without the mare responding aggressively.

Results

Lactation

In the first year, 22 mares were given treatment with domperidone, progesterone and estrogen. Twenty of these mares produced a milk supply deemed adequate to adopt a foal. One of the mares which did not produce adequate milk was a mare with an unknown reproductive history and showed no mammary development during two weeks of hormone stimulation. The other mare did have increased mammary size but produced only a small amount of watery fluid when milked after two weeks of hormone stimulation. One mare was not needed at the time she had achieved a significant amount of milk. This mare was turned out to pasture for two months and then started on the treatment again. At the time she was started on treatment the second time she did not have noticeable mammary development. She responded to treatment after six days and produced adequate milk for a foal. Mares with known histories of multiple prior foals subjectively responded with more milk than those with one prior foal. No differences were noted in mares having foaled the previous year compared to the mares with more time elapsed since the previous foaling. Attempts to induce lactation in April and May were subjectively felt to result in better milk supplies.

In the second year, 17 mares were started on domperidone only after being subjected to artificical photoperiod for a minimum of two months. Sixteen of the second year mares were mares which had been considered successful the year before. Fourteen of these mares responded with an adequate milk supply to adopt a foal. One mare had been added to the herd and did respond to treatment with an adequate milk supply. Two of the mares did not maintain an adequate milk supply for the foals. Domperidone treatment was restarted ten days after adoption in both of these mares. One mare slowly increased her milk supply to acceptable levels over two weeks. The second mare did not recover and throughout this lactation provided only a small amount of milk, approximately half of what was expected. It should be noted that this mare successfully raised a foal the year prior and was deemed to have an excellent milk supply in that year. A single mare which was started on treatment but did not

receive a foal had established an ample milk supply by day 9. No foal was available for this mare and she subsequently stopped producing milk by day 16 of treatment. This occurred in May, so no attempt was made to re-initiate lactation in this mare. This mare is not included in the above numbers.

Adoption

In the first year 16 of the 20 mares used for adoptions adopted their foals fully within one hour. Three of the mares required an additional attempt at nursing one hour after the initial attempt before showing no aggression towards the foal in the stall. A single mare showed favorable signs towards the foal but kicked and squealed when the foal would attempt to nurse. Removal of the foal from the stocks area resulted in a normal maternal response from the mare, with anxious behavior and anxious calling for the foal. Even after these displays she would not allow the foal to nurse. After 12 hours the mare allowed the foal to nurse when twitched. After 24 hours the pair was released in a stall and normal maternal behavior followed. All 20 mares raised their foals until weaning with no differences in maternal behavior compared to normal mares.

In the second year all fifteen mares attempted adopted their foals within one hour after the initial nursing attempt. One mare refused to enter the stocks so the adoption was performed in the stall, with the vaginal stimulation occurring while the mare was entered half into the stall. This mare quickly became interested in the foal and allowed the foal to nurse. For safety reasons this method (outside of the stocks) was not attempted again.

Foals

In the first year the foals grew vigorously after a full milk supply became established in two to four weeks. During the initial weeks following adoption these foals appeared to not gain weight at the same rate as their naturally raised counterparts. By weaning time these foals were indistinguishable from naturally raised foals in the same fields. As yearlings their sizes were as expected based on pedigree and they did not experience a higher than normal incidence of conformation problems or developmental orthopedic problems.

In the second year the foals seemed to take longer until a more normal growth rate occurred, usually four to six weeks before they began to thrive. The two foals whose adoptive dams did not maintain adequate milk supply required supplementation with milk replacer six times daily, supplied by bucket. While these foals should have been getting adequate nutrition from the milk replacer alone they had an unthrifty appearance and were significantly smaller than their peers at weaning. As yearlings they are no longer smaller than their peers and are indistinguishable from other naturally raised yearlings.

Sale results

Sixteen of the first year foals were sold at a major Standardbred auction in November 2009. The results are summarized in Table 1. The average sale price for the farm's consigned horses (n=35) was $25,652. The average sale price for the 16 nurse mare raised foals was $28,188. The average sale price for farm yearlings represented only by the same sires as the nurse mare raised yearlings was $27,620. The farm's highest price yearling at this sale ($67,000) was a nurse mare raised yearling. The prices obtained were within expectations given the sale climate and the pedigrees of the horses offered. The four remaining first year foals were kept for racing or breeding. Race data are not available at this time.

Discussion

The nurse mare needs of this farm were met entirely for two years by induced lactation nurse mares. These mares raised foals which were in every aspect identical to naturally raised foals. The comparable sale prices of the yearlings at auction demonstrate that there is no lasting detriment to the foals when using induced mares.

The goal in the second year was to allow for only oral medications to be used. It was felt that the steroid hormones would be unnecessary in cycling mares. To that end, photoperiod was used alone. Mares were not checked for ovarian activity prior to starting treatment, so it is unknown if this explains the lack of success in getting two of 17 mares to lactate or the failure of two of 15 mares to maintain adequate milk production. The remaining mares performed as expected. Future use of induced lactation mares at this farm will include steroid hormones based on the superior results in the first year, but investigation into the effect cyclicity has on milk production is warranted. An additional concern raised by the poorer response in the second year is the possibility that repeated attempts at inducing lactation in a mare will have diminishing success. This requires further investigation.

In discussion with practitioners cost is often questioned. The additional costs associated with induced lactation mares are the maintenance of the mare for the extra months from weaning until the next foal is adopted and the treatment itself. These costs, on this farm, were less than the cost of leasing a parturient nurse mare from a nurse mare provider. In addition, extra labor and expense are required for parturient nurse mares since most providers

require that the mare be returned in foal. While there is initially increased labor with the induced lactation mares, not having to breed these mares quickly recovers the labor investment. The use of induced lactation nurse mares appears to be a cost saving measure.

The fact that this farm had an established need for nurse mares at known times made this protocol particularly desirable. When a donor mare would begin to show signs of impending foaling a nurse mare would be started on treatment. This usually meant the nurse mare would be eight to ten days into treatment before the foals were ready for adoption. Some mares obtained a level of milk which would have been adequate to supply the foal before the foal was ready for adoption, but in all cases the mare was given a foal within three or four days of being deemed ready. The single mare who did not receive a foal quit producing milk after seven days beyond her peak milk production. It is likely this mare could have been maintained by frequent milking as others have described. Her response, however, does reduce the likelihood that one could start a mare lactating early in the season and hold her ready until she was needed.

While significant numbers are not available to determine if sale results are identical, the lack of noticeable drop in sale price at auction indicates that the animals raised by induced lactation nurse mares are of identical quality to those raised on their own dams. This is important in the adoption of this method of nurse mare production within the horse breeding industry. Although a decline in weight is noted for two to four weeks following adoption, these foals rapidly become equal to foals of the same age. However, information given the author by other practitioners suggests that the initial weight loss is enough to convince owners of valuable animals to not try this technique.

References

1. Daels PF, Bowers-Lepore J: How to induce lactation in a mare and make her adopt an orphan foal: what five years of experience have taught us. Proc Annu Conv Am Assoc Equine Pract 2007. p. 349-353.
2. Steiner JV: How to induce lactation in non-pregnant mares. Proc Annu Conv Am Assoc Equine Pract 2006. p. 259-260.
3. Daels PF,Duchamp G, Porter D: Induction of lactation and adoption of foals by non-parturient mares. Proc Annu Conv Am Assoc Equine Pract 2002. p. 68-71.

Table 1. Public sale results grouped by sire of foal (2009)

	Foals at Sale	Farm Foals at Sale	Nurse Mare Foals at Sale	Total Avg. Sale Price	Farm Avg. Sale Price	Nurse Mare Foal Avg. Sale Price
Sire 1	20	5	4	$19,150	$22,200	$21,500
Sire 2	31	8	5	$30,226	$37,125	$34,000
Sire 3	10	2	1	$20,970	$40,000	$42,000
Sire 4	6	2	2	$32,667	$27,000	$27,000
Sire 5	25	12	4	$16,020	$21,583	$24,750
Total	92	29	16	$23,111	$27,620	$28,187

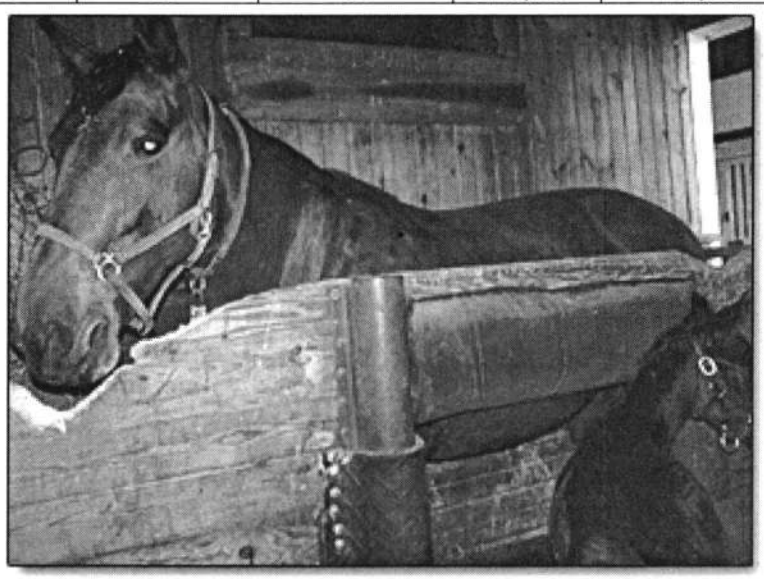

Figure 1. A mare and foal after introduction in the stocks.

Dystocia damage-repair of the mare

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Abstract

Normal equine parturition or dystocia can result in serious trauma to the mare's urogenital or gastrointestinal tract. An understanding of some of the potential problems that can develop is important to consider when relieving dystocias. Equine practitioners should be familiar with the treatment options available for particular injuries. The following proceedings will describe some of the possible injuries that can occur and potential management plan for each injury.

Keywords: Perineal laceration, rectal tear, uterine and vaginal tear, ruptured urinary bladder

Perineal lacerations

Perineal lacerations can occur commonly after parturition or dystocia. There are three degrees of perineal lacerations. First degree lacerations involve the vaginal mucosa. Second degree of perineal lacerations involve the vaginal mucosa and perineal body, but do not involve the rectal-vaginal shelf. Third degree perineal lacerations do disrupt the rectal vaginal shelf and there is communication between the vulva and rectum. This classification does not include rectal-vaginal fistulas which involve a communication between the vulva or vagina and the rectum, but the anal sphincter is not disrupted.

First and second degree perineal lacerations are generally treated conservatively. If a second degree perineal laceration is large enough and has healed by conservative management, a Caslick's technique may be needed to repair the perineal body. If the laceration has disrupted the perineal body extensively, a Gadd technique may be needed to improve the overall conformation of the perineal body. A Gadd technique involves closure of the dorsal vulva after two large triangular flaps of mucosa, which connect dorsally, have been removed. The mucosal flaps can be removed using local anesthesia. The defect created by removing the mucosa is then sutured closed to appose the dorsal aspect of the vulva.

Third degree perineal lacerations generally require surgical repair. The surgical repair is generally performed after the defect has healed conservatively for three to four weeks. In some cases, primary closure just after the defect has occurred may work, but generally fails requiring a second repair three to four weeks later. Once the defect has had time to heal, the mare can be prepared for surgery. Epidural anesthesia is generally performed on typical third degree perineal lacerations and rectal vaginal fistulas. Caudal epidural anesthesia (S6-Cy1 or Cy1-Cy2) is performed using a combination of 100 mg xylazine hydrochloride and 40 mg 2% mepivacaine hydrochloride, diluted with sterile saline solution to make a final injection volume of seven to ten mL. The rectum, vulva and vagina are cleaned of gross contamination using a dilute iodine solution. The method of repair can involve one or two stages. A one stage repair involves complete closure of the rectal-vaginal shelf and perineal body. In a two stage repair, the rectal-vaginal shelf is repaired initially, and then at least three to four weeks later, the perineal body is repaired to complete the second stage. Generally the second stage can be performed using local anesthesia.

Closure of the rectal-vaginal shelf can be performed using either of two methods. In both methods, an incision is made to create a rectal shelf and a vaginal shelf on both the left and right sides. The incision is generally through the demarcation of the rectum and vagina, but should extend more into the vagina as the incision courses caudally. This helps eliminate tension on the final repair. Shelves are created at the cranial aspect of the defect as well. It is important to extend cranially about 2 to 4 cm when creating the shelves in the cranial aspect of the defect, and the shelves on the sides should be undermined so that there is minimal tension on the closure. The shelves can be closed using a simple continuous pattern in a cranial to caudal direction. The vaginal side is closed first in three to four layers followed by the rectal side. Another method involves moving in a cranial to caudal direction using an interrupted six-bite pattern to close both the vaginal and rectal layers together. In the author's experience, the prognosis is generally very good for the repair to stay together. However, we have the advantage of good green grass keeping the feces soft in the spring. It is important to keep the feces soft so that the surgical repair does not dehisce. Mineral oil may have to be given daily to help keep the feces soft in some cases.

Rectal tears

Grade IV rectal tears after parturition are uncommon but require immediate intervention. The rectal tears are believed to develop when gas is trapped within the descending colon and the force of

partition causes the bowel wall to come under extreme tension and tear. Grade IV (full-thickness) rectal tears that communicate with the peritoneal cavity have a poor to guarded prognosis because of the severe peritonitis from fecal contamination of the abdomen. If the tear can be closed efficiently and effectively before severe peritonitis develops, the prognosis could potentially be favorable. Initial management of a parturition rectal tear is similar to iatrogenic rectal tears following palpation.

In the author's practice, we utilize a standing technique to repair the defect within the descending colon. Upon admission mares are sedated with detomidine hydrochloride (0.01mg/kg IV) and butorphanol tartrate (0.02mg/kg IV) and restrained in standing stocks. Complete physical and rectal examinations are performed to evaluate the status of the mare and the severity of the tear. All mares should receive broad-spectrum antibiotics prior to surgical repair. Caudal epidural anesthesia (S6-Cy1 or Cy1-Cy2) is performed, using a combination of 100 mg xylazine hydrochloride and 40 mg 2% mepivacaine hydrochloride, diluted with sterile saline solution to make a final injection volume of seven to ten mL. The rectum is then completely evacuated and the perineal region is aseptically prepared. When an intestinal segment is prolapsed, it is lavaged with copious amounts of sterile saline solution and inspected for viability and traumatic injury, and repositioned through the tear into the abdomen. Once the rectum and caudal descending colon are cleaned of gross contamination, a cotton pack is gently placed cranial to the tear. Care is taken not to enlarge the tear. Stay sutures are placed through the external anal sphincter at the 2, 4, 8, and 10 o'clock positions. The external anal sphincter is transected at the 12 o'clock position to enhance exposure and the rectal tear is retracted caudally using either a finger or a stay suture placed through the caudal margin of the tear. The margins of the tear are inspected and debrided with sterile saline-soaked swabs. Allis tissue forceps are used to accurately appose the margins of the tear then a surgical stapling device (TA-90 or TA-55, 4.8 mm staple length; United States Surgical Corp., Norwalk, CT) is placed below the tissue forceps and then applied. Multiple stapling devices may be needed to completely close the defect. The incision in the anal sphincter can be left open or closed primarily. Abdominal exploration through a midline incision is generally not required, but in select cases involving further injury to a segment of bowel or severe peritonitis more aggressive management may be required.

Post-operatively mares are continued on broad-spectrum antibiotics and nonsteroidal anti-inflammatory agents. Fluid therapy may also be required based on the mare's hydration status. The mares are monitored closely for signs peritonitis and abdominal lavage may be required in some cases. An abdominal drain is placed using a Foley catheter and the abdomen is lavaged using sterile antibiotic solution.

The prognosis varies from case to case and often is based on the degree of abdominal contamination. Initially the prognosis should be guarded, but obviously can change based on how the mare responds following closure of the bowel defect and medical management.

Uterine and vaginal tears

Full thickness tears within the mare's uterus or cranial vagina are serious injuries and can potentially be fatal if not managed aggressively and repaired surgically. In most cases, the full thickness tear is within the gravid uterine horn. Less frequently, the tear is further caudal within the body of the uterus or cranial vagina. Diagnosis is often based on the clinical signs the mare is exhibiting (fever and depression postpartum) along with findings from digital palpation of the uterus or vagina and ultrasound examination. Digital palpation of the uterus is very important in every case to help confirm the diagnosis and determine the location of the tear. Tears in the cranial aspect of the uterus are repaired differently than tears in the caudal uterus or vagina. Ultrasound examination often reveals free fluid within the abdominal cavity. The abdominal fluid can have the characteristics of free abdominal blood. In some cases it can be difficult to determine the difference from hemoperitoneum due to a uterine tear or uterine artery hemorrhage.

Cranial uterine tears are generally repaired through a midline incision. The tear is closed in two layers and the abdomen is lavaged. It is important to check the entire uterus for a second tear. An abdominal drain should be placed in the cranial abdomen prior to closure of the midline incision. The prognosis is generally good if the surgical repair was performed prior to the development of severe septic peritonitis.

Cranial vaginal and caudal uterine lacerations are a potential complication during or after parturition in mares. Vaginal tears can also occur after breeding accidents when the stallion's penis penetrates the cranial vaginal fornix. In cases associated with parturition, immediate clinical signs may

include hemorrhage and bowel evisceration. Surgical repair is indicated to prevent the development of fatal peritonitis. Caudal uterine tears are difficult to reach and adequately repair through a midline celiotomy. Caudal uterine and vaginal tears can be repaired with the mare standing, but repair and secure closure can be difficult. Other problems associated with standing repair include the inability to retract bowel, the complexity of making a multi-layer closure, and the difficulty in securing closure against the weight of the uterus. In the author's practice, we utilize a technique with the mare placed in a Trendelenburg position to easily and efficiently repair most cranial vaginal and caudal uterine tears under general anesthesia. Straps are placed over the hind pasterns, and the mare's hindquarters is elevated above the head using a hoist. In this position, gravity allows the intestines to fall away from the pelvic area.

In cases involving bowel evisceration in pregnant mares, the bowel is replaced into the abdomen after being lavaged with sterile saline, and the fetal position is evaluated. After the fetal position is determined and corrected if necessary, the mare is lowered, and the foal is delivered. The mare is then elevated back into Trendelenburg position for repair of the vaginal and/or uterine tear. Towel clamps are placed in the vulva margin for retraction. To help visualize and illuminate the cranial vagina and caudal uterus, a headlight and portable standing light should be used. The margins of the tear are identified and retracted toward the vulva using either stay sutures or a finger placed in the caudal margin of the tear. Long-handled instruments are needed for secure closure of the tears. The tear is closed in a cranial to caudal direction using an absorbable suture material in a continuous suture pattern. The repair is oversewn again using a continuous inverting pattern to ensure a secure closure. The mare is lowered from the hoist and placed on a padded mat for recovery. The mares are routinely treated with systemic antibiotics and nonsteroidal anti-inflammatory agents. An abdominal drain is placed with the assistance of an ultrasound once the mare is standing. Tears located on the dorsal aspect of the vagina or the dorsal aspect of the caudal uterus are easier to repair. Mares that are treated soon after the tear occurs or before extensive contamination of the abdominal cavity are more likely to survive after surgical repair of the tear.

Ruptured urinary bladder (cystorrhexis)

Rupture of the urinary bladder in postpartum mares is uncommon but should be one of the differential diagnoses in mares that are depressed, febrile, and mildly uncomfortable. Blood work may reveal and elevated or decreased white blood cell count, hyperkalemia, hypochloremia, hyponatremia, and metabolic acidosis. Ultrasound examination will reveal a significant amount of hypoechoic free abdominal fluid. Digital palpation through the urethra may reveal the defect in the bladder or urethra. Endoscopy of the urinary bladder can be used to confirm the presence of a tear within the bladder and/or urethra.

Mares are sedated and a caudal epidural is performed. The perineal region and vaginal cavity are cleaned using a diluted iodine solution. A dorsal speculum is placed and long-handled instruments are used. With the aid of a head light, a small 3 to 4 cm incision is made into the urethra cranial to the urethral sphincter. Using a finger passed through the incision and through the tear, the bladder is intussuscepted into the urethra and exteriorized through the urethral incision. A stay suture keeps the urinary bladder exteriorized during the repair. The defect is repaired using a one to two layer closure with monofilament absorbable suture material. Once the tear is repaired the bladder is repositioned through the urethral incision, and the urethral incision is closed in one or two layers. A urinary catheter is placed for five to seven days. Post-operatively, mares are treated with broad-spectrum antibiotics and nonsteroidal anti-inflammatory agents.

Relationship between donor mare age, semen type, and early embryonic development

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The goal of this retrospective study was to determine if a relationship exists between mare age, semen type (cooled vs. frozen), and early embryonic development in mares. Our hypotheses were that a) embryos collected from mares bred with frozen semen will be smaller than embryos collected from mares bred with cooled semen and b) embryos collected from older mares would be smaller than embryos collected from younger mares.

Donor mares were managed at the Equine Reproduction Laboratory, Colorado State University. Embryo recovery data were included only if the age of the donor mare, semen type (i.e. cooled vs. frozen semen), and date of a single ovulation were known. An embryo flush procedure was performed on day 7 or 8 after ovulation (day 0 = day of ovulation). Embryos were evaluated for morphologic stage, quality, and size. Comparisons of embryo size between groups were made using SAS. All values are presented as the mean ± s.e.m.

Diameter of embryos recovered on day 7 (n=114) from mares bred with cooled semen (401.9 ± 19.6 μm) were larger ($p<0.05$) than embryos recovered from mares on day 7 (n=11) bred with frozen semen (258.2 ± 33.3 μm). Embryos (n=24) collected on day 8 from mares bred with cooled semen tended ($p=0.0553$) to be larger (716.9 ± 104.9 μm) than embryos (n=10) collected on day 8 from mares bred with frozen semen (383.5 ± 54.9 μm). Embryos collected from mares ≤ 5 years of age tended ($p<0.1$) to be larger than embryos collected from mares > 5 years of age. There was no difference in embryo size of embryos collected from mares ≤ 15 or > 15 years of age.

In summary, embryos collected from older mares were not significantly different in size than embryos collected from younger mares. However, size of embryos collected on a given day was affected by semen type, with embryos recovered from mares bred with frozen semen being smaller in diameter than embryos recovered from mares bred with cooled semen. The equine embryo has been reported to enter the uterus between 144 and 156 hours after ovulation. A delay in embryonic development may be associated with a corresponding delay in the time of embryo passage through the oviduct into the uterus. Consequently, it may be advantageous to postpone embryo recovery attempts by 12 to 24 hours for mares bred with frozen semen.

Keywords: Equine, embryo development, embryo transfer, frozen semen

Effects of l-arginine administration on ovarian follicular blood flow

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Perifollicular blood flow has been shown to influence oocyte maturation and, ultimately, potential of the oocyte to fertilize and develop normally in humans and in research animals. Furthermore, mares with significantly higher perifollicular blood flow had higher pregnancy rates following breeding than did mares with low perifollicular blood flow.[1] The hypothesis tested in this research was that administration of the amino acid L-arginine during preovulatory follicle development would increase perifollicular blood flow as evaluated with color Doppler ultrasonography. Twenty-six mares from an embryo transfer recipient herd were used in this study from September to mid-November, 2009. Mares were administered prostaglandin F2α (PGF; 10mg) during the luteal phase and were randomly assigned to receive control vehicle (0.9%saline, i.v.; n=12) or L-arginine (20 gm in 0.9% saline, i.v.; n=14) when the developing follicle was ≥ 30 mm diameter. Treatment was administered in the early morning, and blood flow was evaluated approximately 6 hr later. All mares received treatment on at least two consecutive days, in some cases, (n=3 control; 3 l-arginine) treatment was administered on a third day before ovulation was detected. In all cases, the data reported were from evaluation on the day prior to ovulation detection. For blood flow evaluation, mares were sedated with detomidine hydrochloride and butorphenol tartarate (0.4/0.06 mg., respectively) and were administered butylscopolamine (400 mg) for smooth muscle relaxation. The ovary bearing the preovulatory follicle was examined by color Doppler ultrasonography with an Aloka SSD-2000, (Aloka America, Wallingford, CT). using a finger-mounted 7.5 MHz convex transducer (UST-995-7.5). The percentage of follicle wall with blood-flow color spots was estimated in two-dimensional planes of the entire follicle. The angle of the transducer was varied to display the maximum overall color signals throughout the circumference of the follicle wall. Statistical analysis of the potential difference in perifollicular blood flow was accomplished by least squares one-way analysis of variance with main effect of treatment. Treatment with l-Arginine resulted in greater (p=0.027) perifollicular blood flow compared with control vehicle (percent follicle wall with colored spots, least squares mean ± SEM = 9.6 ± 2.9 vs 19.3 ± 2.9, control vs l-arginine, respectively). Additionally, the data were partitioned into two time periods, with November 1st as the arbitrary cut-off as reproductive response in the recipient herd appeared to exhibit signs of anestrus. Statistical analysis of the partitioned data included main effects of treatment, period and the treatment by period interaction. There was a significant effect of treatment, (p=0.001) and interaction of treatment by period, (p=0.02), suggesting that treatment effect on perifollicular blood flow was lower with time of year. Percent follicle wall with colored spots, (least squares mean ± SEM) was 7.6 ± 3.4 vs 27.8 ± 3.0; control vs l-arginine during September and October, respectively, and 11.6 ± 4.8 vs 10.8 ± 4.8, control vs l-arginine, respectively during November). These data indicate that l-arginine is an effective means of enhancing perifollicular blood flow. Further research is necessary to test the hypothesis that such treatment would lead to higher pregnancy rates or higher quality oocytes.

References

1. Silva LA, Gastal EL, Gastal MO, et al: Relationship between vascularity of the preovulatory follicle and establishment of pregnancy in mares. Anim Reprod 2006;3:339-346

Effect of natural photoperiod on epididymal spermatozoa quality in domestic cat

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The aim of this study was to assess epididymal sperm characteristics in cats under natural photoperiod. The working hypothesis was that natural photoperiod would produce seasonal changes in spermatozoa quality. Epididymides recovered from a program for control of urban feline reproduction at a pet public shelter were used. Toms (n=33) aged between one and five years, were orchiectomized and assigned to one of four groups. Toms were castrated in the two last weeks of spring (SPR, group I, n=10), in the two last weeks of summer (SUM, group II, n=9), in the two last weeks of fall (FAL, group III, n=5), and in the last weeks of winter (WIN, group IV, n=9). Before surgery, all animals were anesthetized with a combination of a ketamine (25 mg/kg i.m.; Vetanarcol®, Laboratorios Koning SA, Argentina), xylazine (1mg/kg i.m.; Sedomin®, Laboratorios Koning SA, Argentina) and atropine (0.04 mg/kg i.m.; Atropina®, Proagro SA, Argentina). All surgical procedures were performed by a licensed veterinarian and followed approved guidelines for ethical treatment of animals. After bilateral orchiectomy, each testis with adjacent epididymis were transported to the laboratory in saline solution. Sperm samples were obtained by cutting the cauda epididymides and the following tests were performed on them: motility (MOT, % motile), velocity (VEL, 0-5), total sperm cells (TS, 10^6), vital stain (VS, % alive), acrosome morphology (ACR, % intact; FITC-PSA), plasma membrane integrity (MI, %intact; CFDA-PI) and sperm morphology (SM, % normal). Data were analyzed by ANOVA and two sets of mean comparisons were performed. The first set compared toms castrated in different seasons and the second set compared toms castrated in days with increasing light (IL, 9h 51' to 14h 27' daylight; Group I and Group IV) versus days with declining light (DL, 14h 27' to 9h 51' daylight; Group II and Group III). There were significant differences between season in sperm MOT, VEL, TS, MI, SM, ACR and VS ($P<0.05$). Furthermore, toms castrated during IL had higher sperm MOT, TS and VS (54.53±3.15 vs. 44.35±4.16, $P<0.05$; 14.87±1.59 vs. 8.53±2.04, $P<0.01$; 61.29±2.14 vs. 54.54±2.70, $P<0.05$; respectively) and tended to have higher VEL and SM (3.7±0.10 vs. 3.39±0.14, $P<0.08$; 43.57±3.13 vs. 34.15±4.15, $P<0.07$) compared to toms castrated in DL. No differences were found in sperm ACR and MI (51.59±3.27 vs. 46.26±3.86, $P>0.29$; 60.39±2.81 vs. 65.14±3.69, $P>0.31$) between both groups. In conclusion, our results show changes in epididymal sperm quality with light changes suggesting that photoperiod may be related to seasonal sperm production.

Keywords: Epididymal sperm, tom cat, seasonal reproduction, increasing light.

Effects of a canine gonadotropin releasing hormone (GnRH) vaccination on male llamas

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Introduction: GnRH is produced in the hypothalamus and regulates the secretion of luteinizing hormone, which is necessary for testosterone production. Immunization against GnRH causes declines in testosterone concentration [T] in horses,[1] swine,[2] cats,[3] sheep[4], cattle[5] and dogs.[6] The aim of this study was to determine the efficacy of a canine GnRH vaccine in llamas for immunocastration. The hypothesis was that GnRH vaccination would decrease [T].

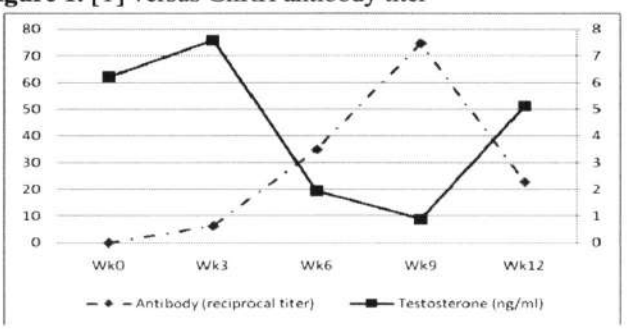
Materials and methods: Mature, intact male llamas received three (3-mL) intramuscular injections of either Canine Gonadotropin Releasing Factor Immunotherapeutic® vaccine (Pfizer Animal Health, Exton, PA; n=7) or an equal volume of sterile diluent provided by the vaccine manufacturer (placebo; n=7) at three week intervals. Jugular venous blood samples were collected every three weeks for 12 weeks. Serum GnRH antibody titers were determined by an enzyme linked immunosorbent assay. Serum [T] were measured using a double antibody radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The assay sensitivity was 0.04 ng/mL. Intra-assay and inter assay CVs were <10%. All of the samples were tested within one assay.

Results: No GnRH antibodies were detected at the time of vaccination or at any later time point in the placebo-treated llamas. Low GnRH antibody titers (1:16-1:256) were detected at 3, 6, 9 and 12 weeks in 2, 5, 6, and 4 of the vaccinated males, respectively. In vaccinated males, mean [T] were inversely related to the geometric mean GnRH antibody titer (Figure 1). However, mean [T] did not differ significantly between treatment groups at any time point.

Conclusion: The response to this GnRH vaccination protocol was an inconsistent, short-lived, modest humoral response resulting in minimal androgen suppression. The reason for different responses to immunization among llamas is not clear. Further investigation on the effects of dose and frequency of immunization is needed.

Keywords: Gonadotropin releasing hormone, immunocastration, llama, testosterone, vaccination

Figure 1. [T] versus GnRH antibody titer



References:

1. Turkstra JA, van der Meer FJUM, Knapp J, et al: Effect of GnRH immunization on sexually mature pony stallions. Anim Reprod Sci 2005;86:247-259.
2. Zamaratskaia G, Rydhmer L, Andersson K, et al: Long-term effect of vaccination against gonadotropin-releasing hormone using Improvac™, on hormonal profile and behavior of male pigs. Anim Reprod Sci 2008;108:37-48.
3. Levy JK, Miller LA, Crawford C, et al: GnRH immunocontraception in cats. Theriogenology 2004;62:1116-1130.
4. Kiyma Z, Adams TE, Hess BW, et al: Gonadal function, sexual behavior, feedlot performance, and carcass traits of ram lambs actively immunized against GnRH. J Anim Sci 2000;78:2237-2243.
5. Cook RB, Popp JD, Kastelic JP, et al: The effects of active immunization against GnRH on testicular development, feedlot performance , and carcass traits of beef bulls. J Anim Sci 2000;78:2778-83.
6. Pfizer Animal Health. Canine Gonadotropin Releasing Factor Immunotherapeutic® vaccine package insert. Exton, PA. #75-0823-03.

Whole blood selenium concentrations in pre-suckle newborn foals

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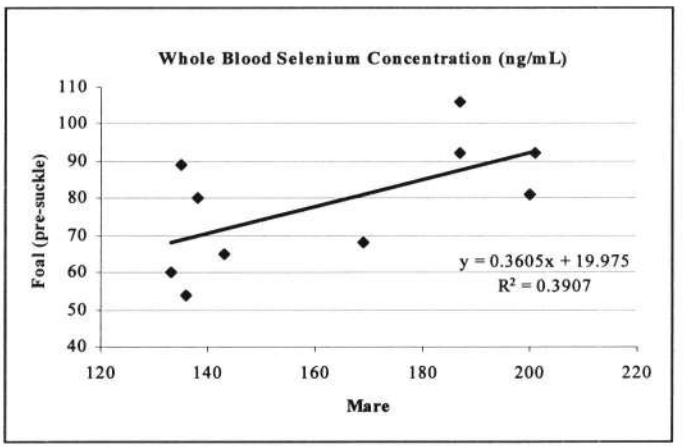
Introduction: Selenium deficiency will cause abortion in pregnant mares and neonatal problems in foals. While most horse owners provide oral selenium supplementation to pregnant mares, losses still occur. The objective of this study was to determine whole blood selenium concentrations in newborn foals from mares that were supplemented with selenium during gestation. The hypothesis was that each foal's whole blood selenium concentration would be similar to that of it dam.

Materials and methods: Ten healthy Quarter Horse mares from the same farm were fed a diet consisting of second cutting alfalfa hay and wet COB with a selenium supplement throughout gestation. Each mare was fed 4-6 mg of selenium (sodium selenite) daily per NRC recommendations. After foaling but before nursing, a whole blood sample was collected from the jugular vein from both the mare and the foal into separate EDTA vacutainer tubes. Samples were shipped on ice to the University of Michigan Veterinary Diagnostic Laboratory, where selenium concentrations were determined. The laboratory's reference range for whole blood selenium concentrations in adult horses is 160-275 ng/mL. The laboratory does not have a reference range for newborn horses but reference values are age dependent and concentrations that are marginal for adults may be adequate for weanlings. Comparisons were made between normal and deficient whole blood selenium concentration groups using a two-tailed Student's t test. Significance was defined as $P<0.05$. Linear regression analysis was used to compare maternal and newborn selenium concentrations. Statistical analysis was performed using Microsoft Excel® software.

Results: Five mares had whole blood selenium concentrations below the reference range (mean±SD137±3.8 ng/mL), which was significantly lower than the other five mares (mean±SD188.8±12.97 ng/mL). All ten foals had very low whole blood selenium concentrations (54-106 ng/mL) that did not differ significantly depending upon which group their dams were in. There was no correlation between each dam's and foal's whole blood selenium concentration (see adjacent figure).

Conclusion: This is the first study to report on whole blood selenium concentrations in pre-suckle newborn foals. Additional data are needed to determine what the normal whole blood selenium concentration is in newborn foals and if NRC recommendations in pregnant mares should be increased.



Keywords: Foal; NRC; pregnant mare; selenium concentration; whole blood

Ovarian color-Doppler ultrasonography to predict ovulation in the bitch

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A non-invasive and rapid, technique to detect the ovulation day in real time for bitches is desirable to determine optimum breeding time. The objective of the study was to determine if color-coded Doppler ultrasonography could be used to identify the ovulation day in the bitch. The hypothesis was that blood flow in the ovary would increase at ovulation and be detected by color-coded Doppler ultrasonography. Ten adult, Walker-type hound (~20 kg) bitches housed at the Louisiana State University School of Veterinary Medicine had a single subcutaneous deslorelin implant (Ovuplant, Wyeth Animal Health, Guelph, Ontario, CA) inserted into the vulvar mucosa to induce estrus. Seven of the ten had normal estrous cycles that were subsequently followed. The estrous cycles were monitored by vaginal cytology and serum progesterone every other day until day one of cytologic diestrus. After the vaginal cytology was 100% cornified, trans-abdominal color-coded Doppler ultrasonography was performed daily, by the same operator, using a 7.5 MHz linear transducer (My Lab50, Esaote, Universal Ultrasound, Bedford Hills, NY, USA) to determine intra-ovarian blood flow. Progesterone values were not known during ultrasonography. A subjective scoring system for quantification of ovarian vascular perfusion was used (1 to 4). The LH surge was assumed to have occurred on the day that progesterone first exceeded 2.0 ng/mL and was considered day zero, with ovulation assumed to occur two days after the LH surge. From a total of 84 ultrasonographic examinations, images were obtained 84.5% and 83.3% of the time of the left and right ovaries, respectively. PROC MEANS, PROC FREQ, and PROC CORR were used for the analysis (SAS version 9.1, SAS, Cary, NC). The relationship between days after the LH surge and the score was explored using Spearman's rank correlation for each dog. The overall significance of the relationship was explored using Cochran-Mantel-Haenszel methods, stratifying over dogs. Significance was considered at $P < 0.05$ for all data unless noted otherwise. The homogeneity of scores across -1 d to +2 d was further analyzed using a Chi square analysis. Where there was significance, pair-wise comparisons between days were performed using Fisher's exact test with significance determined at $P < 0.02$ to reduce type I error. There was an association of days after the LH peak and the ultrasonographic score for the left ($p < 0.001$) and right ovaries ($p < 0.001$). Differences were found for scores on only the right ovary on -1 d to +2 d ($p < 0.001$) (left ovary $P = 0.20$), -1 d and 0 d ($P = 0.006$), and -1 d and 2 d ($P = 0.014$), with higher scores on 0 d and 2 d than on -1 d. Color Doppler ultrasonography provides complementary information about cyclic changes, showing an increase in ovarian blood flow around ovulation.

Keywords: Ovulation; color Doppler; blood flow; progesterone; vaginal cytology

Acklwledgement

Supported by a grant from Audubon Center for Research of Endangered Species

The effect of thyroid releasing hormone (TRH) on serum thyrotopin (TSH), thyroxine (total and free T4) and triiodothyronine (T3) concentration in the alpaca (*Vicugna pacos*)

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Hypothyroidism has been associated with reproductive disorders in humans and laboratory animals and is often considered in cases of infertility in alpacas. However, appropriate testing protocols for evaluating thyroid function in the alpaca have not been established. We hypothesized that exogenous TRH would stimulate the alpaca pituitary-thyroid axis within four hours. The objective of this study was to measure serum concentrations of TSH, total T4 (T4), free T4 (FT4), and T3 before and after exogenous TRH administration.

Alpacas (eight males and five females) with body condition score of three, unremarkable medical histories, and normal physical examination findings were enrolled. Each animal received an intravenous injection (1 mg/450 kg) of TRH (TRH, 044K5015, Sigma Aldrich Chemicals, St. Louis, MO). Blood samples were collected before (t0), 1hr (t1), 2hr (t2), and 4hr (t4) after administration of TRH. Serum samples were frozen at -28 ^{0}C until assayed. Serum TSH (LKKT1) and thyroxine (LKT45) were measured using the Immlite 1000 (Siemens Healthcare Diagnostics Products Ltd, Los Angeles, CA). Free T4 following dialysis (Antech Diagnostics, Irvine, CA) and T3 (TKT31; Siemens Healthcare Diagnostics Products Ltd, Deerfield, IL.) were measured using radioimmunoassay. The response to TRH stimulation was determined by repeated measurement ANOVA.

Serum concentrations of T4, FT4 and T3 (but not TSH) differed ($P < 0.001$) between males and females; therefore all data were analyzed by sex. In females, serum concentration of T3 (ng/dL) increased ($P = 0.034$) following TRH (t0 = 116 ± 15; t1 = 182 ± 18; t2 = 199 ± 21; t4 = 231 ± 26; mean ± SEM) but T4, FT4 and TSH did not. In males, serum concentration of T3 (ng/dL) increased ($P = 0.001$) following TRH (t0 = 141 ± 10; t1 = 241 ± 28; t2 = 277 ± 26; t4 = 342 ± 33) as did serum concentration of T4 (□g/dL; $P = 0.021$) (t0 = 11.3 ± 1.0; t1 = 12.5 ± 1.2; t2 = 13.5 ± 1.1; t4 = 15.5 ± 1.4). Serum concentration of FT4 ($P = 0.14$) and TSH ($P=0.44$) did not change four hours after injection.

In conclusion, administration of 1 mg/450 kg of TRH IV resulted in a significant increase in serum T3 concentration within one hour in all alpacas and a significant increase in serum T4 concentration within four hours in male alpacas. Longer time intervals may be required to demonstrate significant differences in TSH, FT4 and T4 in female alpacas. These data also show a highly significant effect of sex on measured serum concentration of T4, FT4 and T3 in the alpaca.

Keywords: TRH, thyrotropin, thyroxine, triiodothyronine, endocrinology

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Early pregnancy termination by aglepristone in queens

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The aim of this study was to assess the efficacy of aglepristone to terminate early gestation in queens. The hypothesis was that aglepristone would terminate early pregnancy in queens without side effects. Fifteen healthy queens were maintained under artificial illumination (14 h light: 10 h dark) in cages, and 20 days after first mating pregnancy was confirmed by transabdominal ultrasonographic examination using an ultrasound scanner equipped with a 5-7.5–10 MHz linear transducer (Mindray™, DP-6600 Vet; Nanshan, China). On days 21 to 22 of pregnancy, queens were divided in two groups. One group received 10 mg/kg aglepristone (Alizin®, Virbac, Germany, ALI, sc, n=10) on two consecutive days, and the other group received 1 ml of saline solution (PLA, sc, n=5) on two consecutive days. After pregnancy diagnosis females were monitored by ultrasonography until day 10 post-treatment in both groups and weekly until parturition in the PLA group. During each ultrasound examination, the length (LEN), anterior-posterior (AP), and width (WID) dimension of each gestational sac (GS) were measured. The GS volume (GSV) was calculated using the ellipsoid shape formula and GS diameter (GSD), was calculated as the mean of the three measurements of the GS. Data were analyzed with ANOVA using the SAS® program. The pattern of LEN, AP, WID, SD, GSD (mm), and GSV (mm^3) growth of the GS after treatment in ALI group was different compared to PLA group (interaction of treatment by day of treatment, $P<0.001$). On day 0, the LEN, AP, WID, GSD, and GSV growth of the GS were similar in ALI and PLA groups ($P>0.23$). In contrast on day 4-5 and on day10 the LEN, AP, WID, GSD (mm), and GSV (mm^3) of GS were higher in the PLA group compared to the ALI group ([day 4-5, LEN: 25.9±1.2 vs. 18.9±1.0; AP: 18.1±0.8 vs. 12.2±0.7; WID: 21.0±0.9 vs. 16.8±0.9; GSD: 21.6±0.8 vs. 16.1±0.7; GSV: 5053±373 vs. 2457±336; $P<0.01$], [day 10, LEN: 31.3±1.6 vs. 17.2±0.9; AP: 20.0±1.4 vs. 11.9±0.8; WID: 23.9±2.1 vs. 12.7±1.1; GSD: 24.7±1.2 vs. 13.9±0.6; GSV: 7635±670 vs. 1510±358; $P<0.01$]). In the ALI group, gestation interruption occurred in all queens within 4.3±0.6 d after first treatment, whereas in PLA group all queens littered 2.8± 0.3 kittens after a normal gestation period of 64.0±1.0 d. Furthermore, the treatment-interruption of gestation interval was shorter than the treatment-parturition interval (4.3±0.6 vs. 43.0±0.9, $P<0.01$). However, the interval from interruption of gestation to the next estrus and the interval from parturition to next estrus were similar in ALI and PLA (62.8±9.5 vs. 76.2 ± 13.4 d, $P>0.43$). Mean interval between administration of aglepristone and beginning of vaginal discharge was 7.4 ± 0.9 d. It was noteworthy that none of the queens had any clinically detectable side effects during treatment. In conclusion, the use of aglepristone in queens at days 21-22 of pregnancy induced termination of pregnancy without side effects and it could be readily monitored by transabdominal ultrasonography.

Keywords: Queen, aglepristone, interruption of pregnancy, early pregnancy, ultrasonography.

Acknowledgments:

Royal Canin provided the cat food and InMed SRL, provided the ultrasound equipment.

Toll-like receptor-2 mRNA expression in the endometrium of mares resistant and susceptible to endometritis

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Toll-like receptor 2 (TLR-2) is a key regulator of the inflammatory cascade resulting from bacterial infections, and may play a role in equine endometritis. Our objective was to compare TLR-2 mRNA expression in the endometrium of mares resistant and susceptible to endometritis; we hypothesized it would differ between these two categories of mares during physiologic estrus cycle, after insemination with seminal plasma or sperm, and after intra-uterine challenge with *Streptococcus equi* subsp *zooepidemicus (Strep)*.

Mares were evaluated for susceptibility to endometritis using the standard *Strep* challenge model.[1] Accordingly, ten resistant (R) and seven susceptible (S) mares were enrolled. Each mare was used in five cycles (random cross-over design): 1) estrus; 2) diestrus; 3) anestrus; 4) 24 h post-seminal plasma infusion; 5) 24 h post-sperm infusion; with an untreated rest cycle between experimental cycles. In each cycle, a uterine low volume lavage (for culture and cytology) and endometrial biopsy were performed. Individual endometrial tissues were stored frozen in liquid nitrogen until further processing. For analysis, endometrial tissues were thawed, lysed, and mRNA was extracted. The mRNA was processed into cDNA and real-time polymerase chain reaction (RT-PCR) was performed for each sample in duplicate for TLR-2 and glyceradlehyde-3-phosphate dehydrogenase (normalizing gene, GAPDH).[2]

Endometrial TLR-2 mRNA expression differed in R mares between *Strep* and seminal plasma ($p<0.04$), sperm ($p<0.02$) and estrus ($p<0.01$); and in S mares between *Strep* and seminal plasma ($p<0.02$). Our findings indicate that R and S mares both respond to *Strep* infection. However, no differences in TLR-2 tissue expression were found between R and S mares that could account for their differing responses to intrauterine infection with *Strep* or after insemination therefore disproving our hypothesis.

Keywords: Toll-like receptor-2, endometritis, real-time PCR, mRNA, mare

References

1. Hughes J, Loy R: Investigations on the effect of intrauterine inoculations of *Streptococcus zooepidemicus* in the mare. Proc Annu Conv Am Assoc Equine Pract 1969. p. 289-292.
2. Singh Suri S, Janardhan KS, Parbhakar O, et al: Expression of toll-like receptor 4 and 2 in horse lungs. Vet Res 2006;37:541-551.

The use of a simplified hormone protocol for nonovulating embryo recipient mares

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Nonovulating, hormonally-treated mares have been used successfully as embryo recipients. However, most hormonal treatments aimed to suppress ovarian activity in embryo recipients require intensive management and frequent administration of hormones. Our hypothesis was that nonovulating recipient mares receiving single treatments of steroids could be successfully used as embryo recipients. Our objective was to compare pregnancy rates after the transfer of embryos into ovulating versus nonovulating mares receiving one injection of estradiol followed by one injection of progesterone. Embryos were obtained from donor mares (n=40) enrolled in a commercial embryo transfer in Texas, between the months of February and August. When in estrus, donor mares received 1.5 mg of deslorelin intramuscularly (i.m.) to induce ovulation and ovulation was confirmed by ultrasonography (Day 0). All embryos were collected on Day 8 post-ovulation. Embryos collected between February and the first week of April were transferred into nonovulating recipients. Within 72 h of the donor's mare ovulation, nonovulating recipient mares with follicles ≤20 mm and without a corpus luteum received 10 mg of 17β estradiol, i.m.. Approximately 48 h later, mares with significant endometrial edema received 1.5 g of progesterone, i.m. Ovulating mares were used as recipients for embryos collected between April and August. Ovulating mares in estrus received 1.5 mg deslorelin, i.m., to induce ovulation. After collection, embryos (n=90) were transferred non-surgically into the uterus of ovulating (n=47) and nonovulating (n=43) recipients. Nonovulating recipients received embryos between three and eight days after the progesterone treatment. Ovulating recipients were used between Days 3 and 7 post-ovulation. All recipients (ovulating and nonovulating) were maintained on weekly administrations of 1.5 g of progesterone, from the time of transfer until 120 days of gestation. Pregnancy was diagnosed by ultrasonography on Days 12 and 50 post-ovulation and rates were compared using Fisher's exact test, with P-value less than 0.05 considered significant. There were no significant differences in pregnancy rates between ovulating and nonovulating recipients either on Day 12 (43/47, 91% vs. 36/43, 84%) or Day 50 (42/47, 89% vs. 31/43, 72%), respectively. Pregnancy rates in nonovulating recipients were significantly higher when embryos were transferred between Days 3 and 6 (33/36, 92%) versus Days 7 and 8 (3/7, 43%) after progesterone treatment. In conclusion, nonovulating mares can be successfully used as embryo recipients following a single administration of 17β estradiol and progesterone. High pregnancy rates were obtained when transferring embryos into nonovulating recipients between three and six days after progesterone treatment. The hormone protocol presented in this study is beneficial to commercial embryo transfer programs because it reduces the need for frequent reproductive examinations of recipient mares and allows synchronization of a smaller number of recipients per donor mare.

Keywords: nonovulating, synchronization, mares, embryo transfer.

Use of a commercial GnRH vaccination for mismating in bitches

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The objective of this study was to evaluate the use of a commercial GnRH vaccine in bitches to cause luteolysis as a potential mismating treatment. The hypothesis was that vaccination would cause GnRH antibody production resulting in diminution of LH and subsequent luteolysis. Nine bitches were vaccinated with an anti-GnRH (cGRF)vaccine (1 mL SQ, Canine Gonadotropin Releasing Factor Immunotherapeutic®, Pfizer Animal Health, New York, NY) after confirming ovulation based on a progesterone level of >5 ng/mL (Day 0). A booster vaccination was given on Day 14. Blood was collected via jugular venipuncture on days 0, 14, and 28. Progesterone levels were evaluated on Day 0 using a chemiluminescent enzyme immunoassay (Immulite®; Siemens Medical Solutions Diagnostics, Deerfield, IL) and on samples from days 14 and 28 using radioimmunoassay (Coat-A-Count Progesterone In-vitro Diagnostic Test Kit, Diagnostic Products Corporation, Los Angeles, CA). Chemiluminescent enzyme immunoassay was used on Day 0 so same-day results could be obtained for timely vaccination. Progesterone levels confirmed the presence of functional luteal tissue on day 14 and luteolysis (indicated by progesterone levels <2.0 ng/mL) had occurred in all cases by Day 28 ($p=0.0020$). Interestrous intervals were not affected by vaccination. Side effects of vaccination were minor and involved mild erythema at the injection site which resolved without treatment. In addition to these nine bitches, a tenth bitch presented to the Virginia-Maryland Regional College of Veterinary Medicine one day after a mismating occurred. Her progesterone level was 5.0 ng/ml. She was vaccinated with 1 mL SQ of cGRF followed by a booster 14 days later. At 32 days, an ultrasound examination confirmed she was not pregnant and her progesterone level was 0.565 ng/ml. Based on these findings, the cGRF vaccine appears to be useful as an alternative to current mismating treatment options without significant side effects. This study is on-going and subsequent estrous cycles are currently being evaluated to determine if bitches have ovulatory estrous cycles following vaccination.

Keywords: Mismating, luteolysis, canine, GnRH, vaccine

Low dose prostaglandin $F_{2\alpha}$ for luteal regresssion in the bitch

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Commonly used doses of prostaglandin $F_{2\alpha}$ (PGF) for pregnancy termination in dogs (0.15–0.2 mg/kg) are associated with side effects including vomiting, defecation, hypersalivation, tachycardia and anorexia. We hypothesized that multiple, low doses of PGF will cause luteal regression without causing undesirable side effects. A protocol using low dose PGF to cause luteal regression and terminate pregnancy was evaluated in ten Walker-type hound bitches (~20 kg) housed at the Louisiana State University School of Veterinary Medicine during 14 diestrous periods. During diestrus or pregnancy (> 30 days) three pregnant and seven non-pregnant bitches were treated with 0.012 mg/kg SC of PGF (Lutalyse®, Pfizer Animal Health, New York, NY) (n= 10; PGF group) four times a day for five days or until pregnancy was terminated or 1.0 mL SC of 0.9% NaCL solution (n=4; CON group) four times a day for five days. Serum progesterone was measured each morning prior to treatment (AIA-360, TOSOH Bioscience Inc, San Fransisco, CA). After each treatment, bitches were visually observed ~15 min for presence of side effects. Transabdominal ultrasound was used in the pregnant bitches to confirm fetal death and evaluate uterine contents. The difference in progesterone concentration (mg/mL) between days 1 and 3 for the PGF and CON groups were analyzed by a Student's t test (SAS 9.1, Cary, NC) with significance set at $p<0.05$, including Satterthwaite's method for sample populations with unequal variances. Mean (± SD) serum progesterone concentrations difference (decrease) between day 1 and day 3 ($17.1 \pm 10.6 - 1.5 \pm 1.3 = 15.6 \pm 10.6$) of the PGF group was different ($p<0.05$), compared to the difference between day 1 and day 3 ($10.0 \pm 7.3 - 7.6 \pm 2.0 = 2.4 \pm 5.6$) of the CON group. A mild brownish vaginal discharge was observed starting on day 2 to 3 and became abundant on days 4 and 5. Ultrasound examinations on day 3 revealed fetuses with heartbeats, however by day 5 no fetuses or fluid were observed within the uterus. Only one of the bitches in the PGF group showed a side effect, which consisted of a mild tachypnea that subsided within 15 min. The hypothesis that low dose PGF (0.012 mg/kg SC) causes luteal regression with minimal or no side effects was supported, and is a good alternative for pregnancy termination in bitches >30 days of gestation.

Keywords: Prostaglandin, progesterone, luteal regression, pregnancy

Acknowledgement

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Effect of osmolality dilution on motility of frozen thawed equine spermatozoa

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The longevity of osmotically challenged frozen/thawed spermatozoa is postulated to be inhibited in vivo. This study was conducted to evaluate the gradual reduction of thawed semen osmolality on motility over time. The null hypothesis of this study was that a progressive reduction of osmolality of post-thaw spermatozoa would not influence the longevity of the spermatozoa.

Semen was collected from three stallions, housed within 10 km of Wagga Wagga, NSW, and transported fresh to Charles Sturt University, Veterinary Clinical Centre, for analysis and cryopreservation. Semen was extended with EquiPro™ (MiniTüb, Tiefenbach, Germany) and centrifuged prior to the addition of freezing media (5 % glycerol) and processed utilizing standard laboratory protocols, with a controlled freezing mechanism (Freeze Control®, CryoLogic, Mulgrave, VIC, Australia). Straws were thawed in a water bath at 37 °C for 30 sec. Semen from the Control straw was placed in a pre-warmed 37 °C microcentrifuge tube. Semen osmolality was determined utilizing a Fiske 210 Micro-Osmometer (Fiske Associates, Norwood, MA) and progressive individual motility was visually analyzed as a percentage of spermatozoa. Test dilutions were based on EquiPro™ containing varying amounts of analytical grade glycerol (Chem-Supply, Gillman, SA, Australia). Dilution one, total volume of 1 mL, contained 2.5 % glycerol and had a target osmolality of 730 mOsm/kg. Dilution two, total volume of 1 mL, contained 1.25 % glycerol and had a target osmolality of 530 mOsm/kg. Dilutions three and four contained EquiPro™ only (315 mOsm/kg). However, dilution three equalled 1 mL and dilution four equalled 3 mL. Thawed semen, 0.5 mL, was placed in dilution step one, pre-warmed to 37 °C, and allowed to equilibrate at 37 °C for 5 min, prior to being analyzed for osmolality and motility. The subsequent dilutions were added to the total volume of the previous dilution following the same protocol. Once the dilution steps were complete, both Control and Test semen were placed at 22 °C for the remainder of the study and analyzed for motility every hour for 7 hrs and then at 24, 27, 30, 36, and 48 hrs post-thaw.

Results (Figure 1) show that serial dilution of frozen/thawed spermatozoa to a more isosmolal level significantly increases the motility of spermatozoa for the period between 4 and 30 hrs post-thaw in comparison to the Control sample. Further studies could investigate if this thawing technique leads to improved fertility after the insemination of frozen-thawed equine semen.

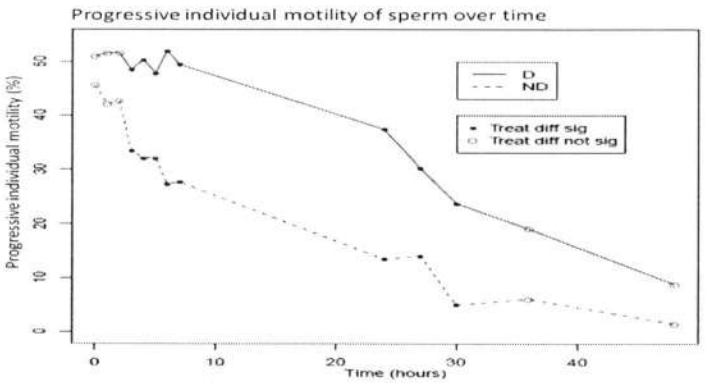

Figure 1 – Plot of motility means for each assessment time. Solid plot-points indicate significant differences between treatments at that assessment time.
D = Dilution (Test) sample
ND = Control sample

Keywords: Osmolality, spermatozoa, motility, cryopreservation, longevity

Differences in uterine canine β-defensin 1 expression during different stages of the estrous cycle

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Pyometra, a prevalent uterine infection that affects intact middle-aged bitches, is typically associated with *E. coli* bacteria. In a disease model we have shown that dogs are particularly susceptible to inoculation of *E. coli* during diestrus, when uterine tissues are under the influence of progesterone. Inoculation in estrus or anestrus did not result in pyometra.
Our hypothesis is that differences in innate immunity account for this differential sensitivity.

The objective of this study was to investigate the differences in gene expression of the antimicrobial peptides β-defensins 1 and 103 as well as the proinflammatory chemokine interleukin-8 (IL-8) in the dog uterus under two conditions: i) during different stages of the estrous cycle and ii) in response to pathogenic *E. coli.*

Twelve post-pubertal, ovariectomized greyhound bitches were treated with estradiol benzoate (Intervet, Bendigo East, Vic, Australia) at a daily dose of 0.6 to 4.8 μg/kg, im, for 13 days. This was followed by 2 mg/kg megestrol acetate (Jurox Pty Ltd., Rutherford NSW, Australia) orally, once a day for 3 days or 16 days to simulate estrus or diestrus, respectively. Uteri were obtained either on day 4 of simulated estrus or on day 10 of simulated diestrus (n=4 per group). Untreated animals served as anestrus controls (n=4). Punch biopsies were obtained and incubated in Dulbecco's Modified Eagle Medium at 37 °C for 0, 3, 5, or 8 hours either with or without a pathogenic *E. coli* strain. Biopsies were then transferred into RNAlater® (Applied Biosystems, Scoresby, Vic, Australia). RNA was extracted and cDNA generated. Using quantitative PCR we demonstrated a 100-fold increase in canine β-defensin 1 mRNA expression during diestrus when compared to anestrus or estrus (at 0 hour time point; $p<0.0001$; as per one way analysis of variance with Bonferroni correction for multiple comparisons). Canine β-defensin 1 expression remained unchanged during incubation with or without bacteria over the 3 to 8 hour time points. Expression levels of IL-8 and canine β-defensin 103 were not significantly different in the different stages of the estrous cycle.

These studies show that expression of the antimicrobial peptide canine β-defensin 1 is hormone-dependent, interestingly with highest-levels corresponding to the stage (diestrus) at which the uterus is most susceptible to infection. Furthermore, this expression does not change in response to *E. coli*, suggesting a constitutive regulatory mechanism.

Studies now in progress will further characterize additional changes in uterine gene expression patterns associated with increased sensitivity during diestrus. A particular focus will be on possible age-related differences in canine β-defensin 1 expression and differences in bitches affected by pyometra.

Keywords: Dog, uterus, defensin1; CBD1, innate immunity; *E. coli*

Effect of corpus luteum and location on pregnancy rate following embryo transfer in alpacas (*Vicugna pacos*)

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In camelids, the corpus luteum (CL) is the sole source of progesterone during pregnancy and its presence is required throughout gestation.[1] Several factors play an important role in the success of an embryo transfer (ET) including the quality of the CL. We hypothesized that the CL size of the recipient at the time of the ET could play an important role in the establishment and maintenance of the pregnancy.

Embryos were collected non-surgically on day 7.4 post-breeding and transferred to synchronized females from a pool of recipients induced to ovulate by buserelin (8.4 µg, IM, Conceptal®, Intervet, Lima, Peru) to match ovulation time with the corresponding donor. Recipients were examined on the day of the transfer and the CL location and size were determined by ultrasonography. Embryos (n=651) were transferred non-surgically into the left uterine horn regardless of the location of the CL. Pregnancy diagnosis was performed eight days after transfer and the effect of the location and the size of the CL on the pregnancy rates were analyzed by ANOVA.

The CL was located on the left ovary and right ovary, respectively, in 57.6% and 42.4% of the recipients. The pregnancy rate was significantly different ($P<0.001$) between recipients with the CL on the left ovary (20.3%) compared to those with the CL on the right ovary (12.4%). None of the recipients with a CL diameter of 9 mm or less became pregnant. The pregnancy rate was also very low in females with a CL > 19 mm however the number of animals in this category was too low to provide a statistical analysis.

In conclusion, our results show that the location of the CL in recipients is a very important parameter to take into account when transferring embryos. Recipients should have a CL of between 10 and 19 mm in diameter. More research is needed to compare the transfer of the embryo to the uterine horn ipsilateral to the CL bearing ovary.

Keywords: Camelid, embryo, pregnancy, maternal recognition

Reference

1. Sumar J: Removal of the ovaries or ablation of the corpus luteum and its effect on the maintenance of gestation in the alpaca and llama. Acta Vet Scand 1983;83(Suppl):133-141

Evidence of a new hierarchy in kisspeptin signaling in the mare

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Our objective was to assess the hierarchy of kisspeptin regulation of gonadotropin secretion in the mare. Experimental aims were to determine if 1) repeated injection of equine kisspeptide (eKP-10, Treatment 1: 0.5 mg eKP-10 iv every 4 h for 3 d) would elicit a different gonadotropin response than repeated injection of GnRH (Treatment 2: 25 μg GnRH iv every 4 h for 3 d; and 2) repeated injection with eKP-10 effected the gonadotropin response to GnRH (Treatment 3: 25 μg GnRH iv at 0 h, 24 h, 48 h, 0.5 mg eKP-10 iv every 4 h in between for 3 d). Mares 5 to 11 days post-ovulation were used and blood samples drawn every 2 h for 12 to 48 h prior to treatment, and every 20 min for 2 h after daily treatments. For all treatments, there was a decrease in LH AUC, and peak response by day three. Basal LH decreased over treatment period ($P=0.003$) for Treatments 1 and 3. LH AUC and peak response was always greater to GnRH than to eKP-10 in Treatment 3 ($P<0.01$). AUC and peak FSH response decreased by day 3 for Treatments 2 and 3 ($P=0.001$), but no change was noted in basal FSH in any treatment. Mares treated with eKP-10 demonstrated a decrease in FSH AUC response ($P=0.02$), but no change in peak response to eKP-10 by day three. These data suggest eKP-10 regulation of LH, in a GnRH independent fashion, directly at the anterior pituitary gland in diestrous mares.

Keywords: Kisspeptin, mare, FSH, LH, progesterone

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Prostatitis with abscessation in a castrated dog

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Canine prostatic disease is common in older dogs (>six years).[1] Reproductive status significantly influences the frequency of certain lesions.[1] Prostate carcinoma has a predilection for castrated males.[2-4] Benign prostatic hyperplasia (BPH) affects nearly all intact males older than nine years and is not seen in castrated males.[5,6] Benign prostatic hyperplasia predisposes intact males to prostatitis, abscessation, and cysts, whereas these conditions are not reported in neutered male dogs.[7-9]

A seven year-old, castrated male Rhodesian Ridgeback presented with a 23-month history of occasional urinary dripping with intermittent hematuria. The patient was castrated six months prior to presentation and had been placed on repeated courses of ciprofloxacin and doxycycline. Signs abated with antibiotic treatment, but returned upon discontinuation.

Upon referral, transrectal digital palpation revealed a symmetrical, non-painful prostate. Ultrasound showed a normal-sized prostate with a multilobulated hypoechoic region in the right lobe measuring 1.2 x 1.7 cm. Prostatic fluid was collected via manual stimulation of the penis. Cytology of the fluid revealed marked numbers of degenerative neutrophils and culture yielded a marked pure growth of *Staphylococcus intermedius* sensitive to ciprofloxacin. The lesion was diagnosed as a prostatic abscess, unusual given the patient was neutered. This may be explained given that signs of chronic prostatitis predated castration. Treatment recommendations included ultrasound-guided drainage and surgical marsupialization or omentalization, with a good prognosis but with risks of peritonitis and urinary incontinence.[1,10]

The patient was placed on six weeks of ciprofloxacin therapy (5 mg/kg). Upon completion, a prostatic omentalization was performed. A culture of the prostatic exudate at this time was negative. Histopathology of prostatic and lymph node tissues confirmed a prostatic cyst with calcification and a stimulated draining lymph node.

The dog developed mild postoperative urinary incontinence, a common complication to prostatic surgery, which responded well to phenylpropanolamine (1 mg/kg BID, Proin™, Pegasus Laboratories, Inc., Pensacola, FL).[11]

Keywords: Prostatic abscess, prostatitis, prostatic omentalization

References

1. Smith J: Canine prostatic disease: a review of anatomy, pathology, diagnosis, and treatment. Theriogenology 2008;70:375-83.
2. Teske E, Naan EC, van Dijk EM, et al: Canine prostate carcinoma: epidemiological evidence of an increased risk in castrated dogs. Mol Cell Endocrinol 2002;197:251-255.
3. Bell FW, Klausner JS, Hayden DW, et al: Clinical and pathologic features of prostatic adenocarcinoma in sexually intact and castrated dogs: 31 cases (1970-1987). J Am Vet Med Assoc 1991;199:1623-1630.
4. Obradovich J, Walshaw R, Goullaud E: The influence of castration on the development of prostatic carcinoma in the dog. 43 cases (1978-1985). J Vet Intern Med 1987;1:183-7.
5. Berry SJL: Development of canine benign prostatic hyperplasia with age. Prostate 1986;9:336-373.
6. Gobello C, Corrada Y: Noninfectious prostatic diseases in dogs. Compend Cont Educ Pract Vet 2002;24:99-107.
7. Barsanti JA, Finco DR: Medical management of canine prostatic hyperplasia. In: Current Veterinary Therapy. 12th ed. Philadelphia: WB Saunders; 1995. p.1033-1034.
8. Black GM, Ling GV, Nyland TG, et al: Prevalence of prostatic cysts in adult, large-breed dogs. J Am Anim Hosp Assoc 1998;34:177-180.
9. Johnston SD, Kamolpatana K, Root-Kustritz MV et al: Prostatic disorders in the dog. Anim Reprod Sci 2000;60-61:405-415.
10. Freitag T, Jerram RM, Walker AM, et al: Surgical management of common canine prostatic conditions. Compend Cont Educ Pract Vet 2007;29:656-673.
11. Mullen HS, Mathiesen DT, Scavelli TD: Results of surgery and postoperative complications in 92 dogs treated for prostatic abscessation by a multiple Penrose drain technique. J Am Anim Hosp Assoc 1990;26:369-379.

Diagnosis of pyometra in a male horned pygmy goat

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The intersex condition is relatively common in goats, and freemartinism can also be seen with intersexing.[1] Mixoploidy, however, has never been reported in small ruminants; it represents a novel etiology for intersex in goats. In this case, peripheral and somatic karyotypes are in disagreement, illustrating both chimerism and chromosomal mosaicism. As a result, use of cytokinetic and molecular techniques to determine genetic sex abnormalities are becoming more important with breeding programs to recognize and eliminate sexual anomalies.

A six-year-old male horned pygmy goat was referred with a history of dysuria and decreased appetite. Prior history included unilateral cryptorchidism and urinary bladder marsupialization. Digital examination of the bladder revealed no obstruction or abnormalities. Upon transabdominal ultrasound, free hypoechoic fluid in the abdomen and two fluid-filled structures were evidenced, approximately five centimeters in diameter. Abdominocentesis with methylene blue infusion confirmed bladder integrity but yielded foul-smelling, mucopurulent fluid. Abdominal exploratory was elected to determine the source of the free fluid and structures. At surgery, one liter of fibrinous abdominal fluid was evacuated and a bicornuate uterus was observed, with what appeared to be a gonad at the proximal end of the left horn. The uterus and broad ligaments were dissected and removed, with samples sent for histopathology and karyotyping. Histopathology revealed retained Müllerian ducts, and a gonad lacking germ cells. Cytogenetic analysis of fibroblasts resulted in 60XX/90XXY, and lymphocytes were 60XX/60XY, evidence of suspected chimerism and mixoploidy.[2] To determine the etiology of the abnormal karyotyping, marker analysis of 17 goat microsatellites was performed. Results indicated that the two probable causes involved the oocyte and that either meiosis II was not completed or the second polar body was not extruded. This karyotype abnormality cannot definitively be connected to the retained Müllerian ducts; speculation for their occurrence involves an anti-Müllerian hormone receptor[3] or ligand mutation.[4]

Keywords: Chimerism, karyotyping, mixoploidy, Müllerian duct

References

1. Brasrur PA, Kochhar HS: Inherited sex abnormalities in goats. In: Youngquist RS, Threlfall W, editors. Current therapy in large animal theriogenology, 2nd ed. St. Louis: Saunders; 2006. p 590-594.
2. Oktem O, Paduch DA, Xu K, et al: Normal female phenotype and ovarian development despite the ovarian expression of the sex-determining region of Y chromosome (SRY) in a 46,XX/69,XXY diploid/triploid mosaic child conceived after in vitro fertilization-intracytoplasmic sperm injection. J Clin Endocrinol Metab 2007;92:1008-1014.
3. Meyers-Wallen VN, Lee MM, Manganaro TF, et al: Mullerian inhibiting substance is present in embryonic testes of dogs with persistent mullerian duct syndrome. Biol Reprod 1993;48:1410-1418.
4. Rey R: Anti-Mullerian hormone in disorders of sex determination and differentiation. Arq Bras Endocrinol Metabol 2005;49: 26-36.

Monochorionic twin pregnancy reduction via trans-abdominal ultrasound-guided cardiac puncture in a mare

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Twin pregnancy is a pathological and undesirable condition in the equine species, occurring in 1 to 2% of the population,[1] and which accounts for 6 to 30% of abortions.[2-4] If not addressed, it may result in poor neonatal viability, dystocia, and reduced fertility of foaling mares. Equine twin pregnancies predominantly originate from the fertilization of two oocytes originating from two follicles (dyzogotic), but monozygotic pregnancy may result from cleavage of an embryo resulting from a single ovulation. This is reported as a rare condition in the horse.[5-8] In humans an increased incidence of monozygotic twins has been attributed to assisted reproduction techniques.[9]

A single late morula grade one embryo was transferred to a 4 year old Quarter Horse cross, recipient mare. The mare was maintained on altrenogest (0.044mg/kg) once daily for 120 days beginning on the day of embryo transfer. A single embryonic vesicle was identified by transrectal ultrasound at 11, 14, 20, and 25 days of gestation. At 34 days of gestation, two distinctly separate embryos with detectable heartbeats were visualized within a single chorion (monochorionic). Ultrasound evaluation was subsequently performed once a week to determine the progression of both fetuses. Both fetuses had normal heart rates and increased in size at each examination, however one was approximately two times larger than the other. On day 126 of gestation successful transcutaneous ultrasound-guided reduction was performed by injecting potassium chloride into the heart of the smaller fetus. The mare was treated with sulfamethoxazole/trimothoprim, flunixin meglumine, and altrenogest. On subsequent repeated examinations through day 206, a single viable fetus with a normal heart rate and activity level was observed. Anatomical examination of the placenta and mummified fetus will be performed, and samples will be collected for DNA testing at the time of foaling.

Keywords: Twin pregnancy, monochorionic, transabdominal reduction.

References

1. Ginther OJ. Reproductive biology of the mare. Cross Plains (WI): Equiservices; 1979.
2. Jeffcott LB, Whitwell KW. Twinning as a cause of neonatal loss in the Thoroughbred. J Comp Pathol 1973;83:91-105.
3. Roberts SJ. Physiology of the gestational period. In: Veterinary obstetrics and genital diseases. 3rd ed. Woodstock (VT): Published by the author; 1986; p. 81-106.
4. Giles R, Donahue J, Hong C, et al. Causes of abortion, stillbirth, and perinatal death in horses: 3527 cases (1986–1991). J Am Vet Med Assoc 1993;8:1170-1175.
5. McCue PM, Thayer J, Squires EL, et al. Twin pregnancy following transfer of single embryos in three mares: a case report. J Equine Vet Sci 1998;8:832-834.
6. Govaere J, Hoogewijs M, Schauwer CD, et al. An abortion of monozygotic twins in a Warmblood mare. Reprod Domest Anim 2008;44:852-854.
7. Rooney JR. Autopsy of the horse. Baltimore: Williams & Wilkins; 1970. p. 120-134.
8. Meadows SJ, Binns MM, Newcombe JR, et al. Identical triplets in a Thoroughbred mare. Equine Vet J 1995;27:394-397.
9. Aston KI, Peterson CM, Carrell DT. Monozygotic twinning associated with assisted reproductive technologies: a review. Reproduction 2008;136:377-386.

Atypical presentation of granulosa-theca cell tumor in a broodmare

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Granulosa theca cell tumors (GTCT), originating from differentiated ovarian stroma, are the most common ovarian neoplasm in the mare.[1] Granulosa theca cell tumors have various effects on cyclicity and behavior, depending on the hormones produced. Diagnosis is based upon reproductive history, endocrine profiles, and clinical findings such as transrectal palpation and ultrasonography. Definitive diagnosis is based on histopathological examination.

A 4-year-old maiden Quarter Horse mare, presented to the WSU-VTH theriogenology service for a 14 day pregnancy diagnosis in March 2008. Transrectal ultrasound revealed a cystic mass of 83.8 mm on the left ovary, containing a smaller 35 mm follicular structure. The right ovary was small and inactive. Results of serum endocrine profiling were as follows: testosterone 46.6 pg/ml, progesterone 0.1 ng/ml, and inhibin 0.57 ng/ml. These results were inconsistent with GTCT diagnosis.[2] Repeated examinations demonstrated normal cyclicity of the right ovary with a prolonged interovulatory period and no changes in the left ovary. Due to concerns about permenant changes with possible affects on reproductive success, surgical removal of the left ovary was elected and ovariectomy performed via standing flank laparoscopy. Histopathology confirmed a diagnosis of GTCT. The mare was bred by live cover in February 2009 and pregnancy was diagnosed at 15 days. Progesterone levels were within normal range in the first 40 days. Pregnancy was evaluated at 60 days and again at 10 months.

This case report demonstrates the importance of serial monitoring of ovarian changes and good communication with owners regarding surgical options when a definitive diagnosis cannot be made based on a gold standard diagnostic technique in unusual cases of GTCT. It is critical that the owner understand the limitations of diagnostic techniques so that the best possible outcome may be achieved.

Keywords: Granulosa theca cell tumor, infertility, mare, neoplasia, ovary

References

1. Zelli R, Lakamy S, Maurizio M, et al: Gonadotropin secretion and pituitary responsiveness to GnRH in mares with granulosa-theca cell tumor. Theriogenology 2006;66:1210-1218.
2. Bailey MT, Tredsson MHT, Wheaton JE: Inhibin concentration in mares with granulosa cell tumors. Theriogenology 2002;57:1885-1895.

Recovery of a stallion with a chronic scrotal hydro/pyocele and azoospermia

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Chronic fluid accumulations in the scrotum are often assumed to have a poor prognosis, especially when associated with azoospermia, and often result in castration. A 9-year old Andalusian stallion presented with a one-month history of an enlarged scrotum that was non-responsive to diuretics, anti-inflammatory medication, exercise, and hydrotherapy. The physical examination was within normal limits except for prominent bilateral scrotal enlargement. Testicular ultrasound showed increased testicular blood flow, bilaterally enlarged spermatic cords, and large amounts (4-6 cm) of hyperecoic fluid within the vaginal cavity. Cloudy, opaque, yellow fluid with an increased total protein (5.5 g/dL) and cellularity (WBC: 128,000 cells/μL, RBC: 12,000 cells/μL) was recovered by scrotalcentesis; gram stain and culture were negative. Collected semen contained few immotile sperm. The stallion was diagnosed with bilateral pyocele, but the cause was unknown.

A drain was placed in the right vaginal cavity and flushed for four days before removal. The left vaginal cavity was drained and flushed once without the placement of a drain. Within a week the scrotum returned to a normal size. The stallion was treated with doxycycline, saccharomyces capsules, flunixin meglumine, and frequent exercise.

Two months after discharge the scrotal contents had minimal abnormal changes. The second of two ejaculates contained 7.1 billion sperm with 79/55% total/progressively motile sperm and 38% morphologically normal sperm.

An enlarged scrotum is often associated with trauma or hot summer conditions, but may also be secondary to castration, peritonitis, orchitis, or inguinal hernia.[1,2] In breeding animals the primary complication of an enlarged scrotum is reduced fertility due increased testicular temperature.[2,3] Common treatments include anti-inflammatory medication, hydrotherapy, exercise, and castration of chronic cases.[4,5] Stallions with chronic hydro/pyocele and azoospermia should not be assumed permanently sterile, and in this case drain placement facilitated fluid removal and apparent recovery of testes function.

Keywords: Stallion, scrotum, testes, hydrocoele, pyocoele, azoospermia

References

1. Colbourne CM, Adkins AR, Yovich JV: Hydrocele formation after castration in 3 geldings. Aust Vet J 1996;73:156-157.
2. Morresey PR: The enlarged scrotum. Clin Tech Equine Pract 2007;6:265-270.
3. Shore MD, Bretzlaff KN, Thompson JA, et al: Outcome of scrotal hydrocele in 26 bulls. J Am Vet Med Assoc 1995;207:757-760.
4. Bartholomew AB, Caudle AB, Peroni JF: Unusual clinical presentation of scrotal hydrocele in a Tennessee walking stallion. Compend Contin Educ Pract Vet 2003;25:632-663.
5. Frazer GS: Stallion reproductive emergencies. Proc North Am Vet Conf 2008. p. 106-109.

Surgical correction of priapism in an 18 year old Quarter Horse gelding

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Priapism is a prolonged penile erection in the absence of sexual desire, and is observed in several animal species as well as in man.[1] Though uncommon, priapism in horses is most often observed in stallions and is occasionally reported following administration of phenothiazine derivatives for sedation.[2,3] Impotence is a common sequella to priapism in stallions, making successful treatment critical in valuable breeding animals.[2] Medical management of priapism may utilize slings, ice baths, massage, cholinergic blockers, diuretics and alpha-adrenergic agents.[2,4] If conservative therapy is unsuccessful, irrigation of the corpus cavernosum penis with heparinized saline or surgical procedures such as vascular shunts, partial phallectomy, or complete phallectomy with urethrostomy may be performed.

An 18 year old Quarter Horse gelding presented for apparent inability to retract his penis of less than two hours duration. Upon physical examination, selective erection of the corpus cavernosus tissue suggested the diagnosis of priapism. No other abnormalities were noted. No history of trauma, diet change, or administration of phenothiazine derivatives was reported. Initial treatment included injections of phenylephrine into the corpus cavernosum, and through-and-through heparinized saline lavage of the corpus cavernosum penis. Both techniques resulted in temporary resolution of the priapism. Benztropine mesylate was administered intravenously with no noted improvement. The patient was also treated with penile slings, ice packs, purse string sutures and acupuncture. The priapism remained refractory to medical treatment and ten days after admission, *en bloc* resection and perineal urethrostomy with spatulation of the urethra was performed. The gelding recovered well from surgery, and returned to his previous level of athleticism.

The pathogenesis of priapism in the horse is not well understood, and reports of successful treatment with medical management alone are limited.[3] *En bloc* resection with perineal urethrostomy was a successful means of treatment for priapism in this gelding.

Keywords: Priapism, equine, phallectomy, gelding, *en bloc* resection

References

1. Rochat MC: Priapism: a review. Theriogenology 2001;56:713-722.
2. Boller M, Furst A, Ringer S, et al: Complete recovery from long-standing priapism in a stallion after propionylpromazine/xylazine sedation. Equine Vet Educ 2005;17:305-311.
3. Van Harreveld PD, Gaughan EM: Partial phallectomy to treat priapism in a horse. Aust Vet J 1999;77:167-169.
4. Schumacher J, Varner DD: Surgical correction of abnormalities affecting the reproductive organs of stallions. In: Youngquist RS, Threlfall WR, editors. Current therapy in large animal theriogenology. 2nd ed. Philadelphia: Saunders; 2007. p. 23-36.

Successful pregnancy from artificial insemination after removal of a uterine leiomyoma

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A 16-year-old Thoroughbred mare was evaluated during her foal heat. All vital parameters were within normal limits. Transrectal ultrasound revealed a uterine mass located at the base of the right horn. Sterile saline injected into the uterus did not disperse into the body of the right horn past the mass, and the mass was thought to fill the entire lumen of the right horn. A small cyst was noted in the body of the left horn. Endoscopy of the uterus revealed a large, well encapsulated, lobulated mass and biopsies were collected for histopathology, however the biopsy report was inconclusive. The mare's value rested in her breeding soundness, and she had produced many healthy, valuable foals in the past, so mass removal was elected with an expected return to reproductive function. The mass was removed a week later using laser surgery and manual extraction and consisted of soft tissue with a central area of calcification. Sections were submitted for histopathology, and a diagnosis of uterine leiomyoma was made. The mare was artificially inseminated during the next breeding season following the mass removal with fresh, cooled semen from an on-site stallion. At the time of abstract submission, this mare is 337 days pregnant and awaiting foaling. Uterine leiomyomas are the most common neoplasm of the human urogenital tract, and, while rare in animals, have been reported in cattle, non-human primates, cats, dogs, and sea mammals. Nearly all cases reported of equine uterine masses have involved the right horn and/or the right ovary. The reason for this discrepancy is unknown. Multiple techniques have been described to visualize and remove uterine masses, and a full return to breeding soundness appears to be the most commonly reported outcome.

Suggested reading

Backlin BM, Eriksson L, Olovsson M: Histology of uterine leiomyoma and occurance in relation to reproduction activity in the Baltic gray seal. Vet Pathol 2003:40,175-180.

Barrell E, Hendrickson DA: Rebreeding of the partially hysterectomised mare: uterine surface area as a factor of success. Equine Vet Educ 2009;21:205-206.

Berezowski C: Diagnosis of a uterine leiomyoma using hysteroscopy and a partial ovariohysterectomy in a mare. Can Vet J 2002;43:968-970.

Bracher V, Mathias S, Allen WR: Videoendoscopic evaluation of the mare's uterus: II. Findings in subfertile mares. Equine Vet J 1992;24:279-284

Broome TA, Allen D, Baxter GM, et al: Septic metritis secondary to torsion of a pedunculated uterine fibroleiomyoma in a filly. J Am Vet Med Assoc 1992;200:1685-1688.

Heijltjes JM, Rijkenhuizen ABM, Hendriks WK, et al: Removal by laparoscopic partial ovariohysterectomy of a uterine leiomyoma assumed to have caused fetal death in a mare. Equine Vet Educ 2009;21:198-203.

Hinojosa AM, Holyoak G, Moll D, et al: Theriogenology question of the month. J Am Vet Med Assoc 2003;223:786-788.

Hoffsis GF, Davis GW, Saint-Jean G: Leiomyoma of the uterus of a mare. Can Vet J 1986;27:319-320.

Hurcombe SDA, Slovis NM, Kohn CW, et al: Poorly differentiated leiomyosarcoma of the urogenital tract in a horse. J Am Vet Med Assoc 2008;233:1908-1912.

Janicek JC, Rodgerson DH, Boone BL: Use of a hand-assisted laparoscopic technique for removal of a uterine leiomyoma in a standing mare. J Am Vet Med Assoc 2004;225:911-914.

McCue PM: Neoplasia of the female reproductive tract. Vet Clin North Am Equine Pract 1998;4:505-515.

Muurlink T, Walmsley J, Whitton C: Successful laparoscopic surgery for a uterine leiomyoma in a mare. Equine Vet Educ 2008;20:508-511.

Naiper JE, Murray S, Garner MM, et al: Uterine leiomyomas in three captive eastern bongo (*Tragelaphus eurycerus isaaci*). J Zoo Wildl Med 2005;36:709-711.

Quinn GC, Woodford NS: Infertility due to a uterine leiomyoma in a Thoroughbred mare: clinical findings, treatment and outcome. Equine Vet Educ 2005;17:150-155.

Rodgerson DH, Brown MP, Watt BC, et al: Hand-assisted laparoscopic technique for removal of ovarian tumors in standing mares. J Am Vet Med Assoc 2002;220:1503-1507.

Romagnoli SE, Momont HW, Hilbert BJ, et al: Multiple reoccurring uterocervical leiomyomas in two half-sibling Appaloosa fillies. J Am Vet Med Assoc 1987;191:1449-1450.

Santschi EM: Laparoscopic uterine tumor removal. . . are we there yet? Equine Vet Educ 2008;20:513-514.

Santschi EM, Slone DE:) Successful pregnancy after partial hysterectomy in two mares. J Am Vet Med Assoc 1994;205:1180-1182.

Sendag S, Cetin Y, Alan M, et al: Cervical leiomyoma in a dairy cow during pregnancy. Anim Reprod Sci 2008;103:355-359.

Sweeny RW, Hamir AN, Fisher RR: Lymphosarcoma with urinary bladder infiltration in a horse. J Am Vet Med Assoc 1991;199:1177-1178.

Van Bressem MF, Van Waerebeek K, Siebert U, et al: Genital diseases in the Peruvian dusky dolphin (*Lagenorhynchus obscurus*). J Comp Pathol 2000;122:266-277.

Walker CL, Cesen-Cummings K, Houle C, et al: Protective effect of pregnancy for development of uterine leiomyoma. Carcinogenesis 2001;22:2049-2052.

Retrograde ejaculation in a stallion associated with tail-head trauma

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Low sperm numbers and motility are common in stallions presented with a history of reduced fertility and are oftentimes related to testicular dysfunction[1]. This case presents a similar history but is associated with retrograde ejaculation rather than testicular dysfunction.

A nine year-old stallion was presented with a history of a fluctuation in the total sperm numbers accompanied by poor sperm motility in the previous two breeding seasons. A complete physical examination was normal with the exception of limited tail motion due to complications from a previous tail-block. Testicular size was normal (volume-385cm^3) with a predicted daily sperm output of 8 billion sperm. Two ejaculates were collected in an artificial vagina and contained approximately 400 million sperm in each ejaculate. Spermatozoal motility was 66/45% and 44/28% for total and progressive motility, respectively; and the percent morphologically normal was 78 and 85; respectively, for the first and second ejaculates. The bladder was catheterized and 1700 ml of urine was collected that contained approximately 16.81 billion sperm. A diagnosis of retrograde ejaculation was made.

The stallion was administered oral imipramine, a tricyclic anti-depressant used to treat men with retrograde ejaculation, and pyrilamine maleate and pseudoephedrine HCl (an α-adrenergic agonist) granules (Tri-Hist®, Neogen, Lexington, KY). He returned one week later with little improvement.

Normal copulation results in closure of the urinary bladder neck and is mediated by sympathetic stimulation of α-adrenergic receptors[2]. Because this stallion had noticeable tail head injury, it is possible that damage to these nerves caused his clinical signs . Retrograde ejaculation has been reported in humans,[3,4] dogs,[5] rams,[6] and bulls.[7] Retrograde ejaculation should be considered when stallions are presented for reduced sperm numbers associated with apparently normal testes size.

Keywords: Stallion, retrograde ejaculation, sperm

References

1. Johnson L, Blanchard TL, Varner DD, et al: Factors affecting spermatogenesis in the stallion. Theriogenology 1997;48:1199-1216.
2. Brinsko SP: Retrograde ejaculation in a stallion. J Am Vet Med Assoc 2001;218:551-553.
3. Hershlag A, Schiff SF, DeCherney AH: Retrograde ejaculation. Hum Reprod 1991;6:255-258.
4. Yavetz H, Yogev L, Hauser R, et al: Retrograde ejaculation. Hum Reprod 1994;9:381-386.
5. Beaufays F, Onclin K, Verstegen: Retrograde ejaculation occurs in the dog, but can be prevented by pretreatment with phenylpropanolamine: a urodynamic study. Theriogenology 2008;70:1057-1064.
6 Pineda MH, Dooley MP, Hembrough FB: Retrograde flow of spermatozoa into the urinary bladder of rams. Am J Vet Res 1987;48:562-568.
7. Dooley MP, Pineda MH, Maurer RR, et al: Evidence for retrograde flow of spermatozoa into the urinary bladder of bulls during electroejaculation. Theriogenology 1986:101-109.

Estrus detection in mares using contextually congruent stallion vocalization playback with and without stallion scent

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We evaluated stallion sexual vocalizations with and without olfactory stallion stimuli as an aid to estrus detection in mares. Eleven horse mares were exposed to either auditory or auditory with added olfactory stimuli on a Monday-Wednesday-Friday (M-W-F) schedule for detection of estrus. A contextually congruent three-minute soundtrack of stallion vocalizations was presented to mares at rest in stalls simulating a live stallion approaching from out of view. Preputial smegma samples obtained from the same stallion were diffused into the mare's stall as olfactory stimulus. Frequency as well as latency to the first occurrence for each of six specific receptive and six non-receptive responses were recorded from video recordings of the trials. Additionally, an overall determination of estrus, diestrus, or ambivalent behavior was made. Confirmation of ovarian status was based on records of daily transrectal palpation and ultrasound, along with RIA serum progesterone levels. During the four week study period, for 12 of 15 ovulations, estrus was detected with the M-W-F auditory and olfactory protocol, within zero to 10 days before ovulation (mean 4.4 d, SD 2.7). For 8 of 9 occasions for which study protocol and live stallion estrus detection results were discrepant, results with auditory and olfactory stimuli were more consistent with ovarian status. We conclude that auditory stimuli in the form of recorded stallion vocalizations presented to mares in a contextually congruent manner were at least as effective as a live stallion and in some instances more accurate. Further, the addition of olfactory stimuli increased the intensity of estrus response elicited.

Key words: Estrus detection, mare, vocalizations, olfactory, auditory, stallion

Safety of stallion testicular biopsy performed by novice operators

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Testicular biopsy is a valuable technique for the study of testicular function and diagnosis of testicular diseases. However, this technique remains seldom used in stallions due to fear of complications. The aim of the present study was to evaluate the efficacy and safety of testicular biopsy in stallions performed by novice operators.

Six adult stallions (4 to 7 years old) scheduled for castration were used. Testicular biopsies were obtained aseptically using a 14 ga core self-firing instrument (Bard Biopsy Systems, Tempe, AZ) by senior veterinary students with no prior experience. Students were given instructions in a 30-minute seminar regarding how to perform the technique. Stallions were placed in stocks and sedated with detomidine HCl (10 μg/kg; IV) or xylazine (0.5 mg/kg; IV) and butorphanol tartrate (0.01 mg/kg; IV). Testicular ultrasonography was performed on all stallions before, and daily for five days after the procedure. The marginal part of the testicular artery was evaluated using pulsed-wave color Doppler and the peak systolic velocity (PSV), end diastolic velocity (EDV), resistance index, and pulsatility index were recorded using standard techniques.[1] Measurements before and after biopsy sampling were compared within and between testicles using general ANOVA/AOCV after log transformation. Biopsy samples were evaluated to determine their diagnostic validity. Stallions were castrated ten days after the biopsy procedure and testes were examined grossly for any abnormalities. Representative samples were submitted for histopathological evaluation. All stallions received antimicrobials daily for four days, starting one day before the biopsy procedure. A single dose of flunixin meglumine (1.1 mg/kg; IV) was given following the procedure.

No systemic effects were observed during the study period. Ultrasound evaluations revealed subcutaneous/albuginea hematomas in three stallions, which resolved within one week. After 10 days, the biopsy site was identified on each excised testicle as a pin point. No gross abnormalities were detected except for a small amount of fibrinous material between the albuginea and the tunica vaginalis in one stallion. There was no significant effect of biopsy sampling on blood flow ($P< 0.05$) when paired testicles were compared. There was no difference between biopsied and non-biopsied testicles for PSV ($P=0.39$) and EDV ($P=0.47$). However, there was a significant decrease over days for PSV ($P=0.011$) and for EDV ($P=0.02$). Core testicular biopsy sampling, using a self-firing Bard® instrument, is a safe and reliable procedure in stallions even in the hand of novice operators if they follow simple instructions.

Keywords: Testis, biopsy, histology, pathology, equine

Reference

1. Pozor MA, McDonnell SM. Color Doppler ultrasound evaluation of testicular blood flow in stallions. Theriogenology 2004;61:799–810.

Effects of altrenogest treatment and age of the mare on conceptus growth and secretion of reproductive hormones during early pregnancy

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Embryonic mortality is a major reason for low reproductive efficiency in mares. Treatment of mares with repeated early embryonic loss with the synthetic progestin altrenogest has become routine in stud farm practice. However, no controlled studies on the efficiency of altrenogest administration to prevent embryonic losses and the potential mechanisms behind such treatment are available so far. In the present study, we have investigated effects of altrenogest treatment on conceptus development and the secretion of luteinizing hormone (LH), progesterone and equine chorionic gonadotropibn (eCG) until day 100 of pregnancy in fertile broodmares of two age groups (4-8 years, >8 years). Mares were inseminated with fresh semen using standard procedures and treated orally with altrenogest (0.044 mg/kg once daily, Regumate®, Intervet/Schering-Plough Animal Health, Millsboro, DE) or placebo (sunflower oil, 10 ml once daily) from day 6 to 100 after ovulation. Blood samples were collected throughout the study and size of the embryonic vesicle and embryo/fetus was determined by ultrasound. Statistical comparisons were made with the SPSS statistics package (SPSS, Chicago, IL) by ANOVA using a general linear model for repeated measures. In case of overall significance, values differing from each other were identified by testing for least significant differences. A P-value < 0.05 was considered significant. Data given are means±SEM.

No difference in the per cycle pregnancy rate between altrenogest-treated (75%) and control mares (74%) was detected and all mares pregnant on day 12 after ovulation gave birth to healthy foals the next spring. A significant effect of age group but not altrenogest treatment on diameter of the embryonic vesicle was found on day 15 (control, 4-8 years: 22.9±1.0 mm, >8 years: 22.0±1.7 mm, altrenogest, 4-8 years: 26.1±2.0 mm, >8 years: 20.4±1.0 mm, $p<0.05$). Age of mares also significantly influenced size of the embryo on day 30 ($p<0.05$). Conceptus development is thus inferior in mares aged >8 years compared to mares 4 to 8 years of age. A positive effect of altrenogest on size of the embryo/fetus was detected on days 35 and 40 ($p<0.05$). In the control but not the altrenogest group, size of the embryo respective fetus was negatively correlated with age of the mares (day 30: r=-834, $p<0.05$; day 35: r=-0.506, $p<0.05$). The concentration of LH was neither effected by age nor by treatment of mares. For progesterone concentration, an interaction between day, treatment and group was detected between days 35 and 50 ($p<0.05$). An increase in eCG between day 40 and 49 was neither affected by age or treatment, but on days 79 and 89, a significant interaction of age and treatment ($p<0.05$) indicates a positive effect of altrenogest on eCG secretion in 4 to 8 year-old mares.

In conclusion, the present study demonstrates a positive influence of altrenogest treatment on delayed conceptus development in pregnant mares. This effect did not occur in early pregnancy but during the second critical phase of pregnancy when organogenesis is completed and placentation initiated between days 35 and 40. This justifies altrenogest treatment of older mares or those with a history of early embryonic death to support conceptus development.

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Effect of administration of exogenous oxytocin during diestrus on corpora luteal function and endometrial oxytocin receptor concentration in cycling mares

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We have previously demonstrated that administration of 60 units of oxytocin twice daily on days 7 to 14 after ovulation induced prolonged corpora luteal (CL) function in mares, which may be a plausible method of suppressing estrus.[1] The objectives of this study were to: 1) compare twice versus once daily administration of oxytocin on the duration of CL function and 2) determine the effect of oxytocin treatment on endometrial oxytocin receptor concentration. In experiment 1, jugular blood samples were collected every other day on days 0 (ovulation) to 50 for determination of progesterone concentration. On day 7, mares were randomly assigned to three groups: 1) untreated control (n = 7), 2) BID oxytocin-treated (n = 7) and 3) SID oxytocin-treated (n = 8), and the oxytocin-treated mares received 60 units oxytocin IM twice daily (BID group) or once daily (SID group) through day 14. Mares were considered to have prolonged CL function if progesterone remained >1.0 ng/ml through day 30. One of 7 control, 5 of 7 BID oxytocin-treated and 5 of 8 SID oxytocin-treated mares had prolonged CL function. There was no significant difference in the proportion of mares with prolonged CL function between the two oxytocin-treated groups, and collectively, oxytocin treatment increased ($P<0.05$) the proportion of mares with prolonged CL function compared to no treatment. In experiment 2, mares were randomly assigned to two groups (n = 5/group): 1) saline-treated control and 2) oxytocin-treated. Beginning on day 7, control mares received 3 cc sterile saline IM BID and oxytocin-treated mares received 60 units oxytocin IM BID through day 14. On day 15, approximately 1.0 g of endometrium was obtained transcervically from each mare for determination of oxytocin receptor concentration. There was no significant difference in the oxytocin receptor concentration between control and oxytocin-treated mares (1,465.7 ± 108 and 1,382.8 ± 108 fmol/mg protein, respectively). In summary, once daily administration was as effective as twice daily administration of oxytocin for prolonging CL function, and oxytocin treatment did not alter the concentration of endometrial oxytocin receptors.

Keywords: Equine, mare, oxytocin, corpus luteum, oxytocin receptor

Reference

1. Vanderwall DK, Rasmussen DM, Woods GL: Effect of repeated administration of oxytocin during diestrus on the duration of corpora luteal function in mares. J. Am. Vet. Med. Assoc. 2007;231:1864-1867.

Development and validation of the canine neonatal vitality score (CNVS)

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No *validated* scoring systems, similar to the human Apgar score, for evaluation of newborn puppies exist for either a research or clinical setting. The objective of this study was to develop a validated scoring system that had a Cronbach's alpha of greater than 0.7 and a significant correlation to resuscitation outcome. Initial data were collected from 129 puppies from four clinics and the CNVS was the result. This CNVS scoring system was then re-distributed to four clinics (one differed) and applied to 113 additional puppies. Each category (heart rate, respiratory effort, mucus membrane color, activity and muscle tone, suckle reflex, and lumbosacral stimulation) received a value of 0-2 points for a possible 12 total. Following factor analysis, the CNVS was considered valid with a Cronbach's alpha of 0.93. Convergent validity was tested using correlations to outcome. The total CNVS value was moderately inversely correlated with the length of time the puppy was stimulated ($rho = -0.5803$, $p<0.00001$) and the number of interventions needed to revive the puppy ($rho = -0.6267$, $p<0.00001$). This study demonstrates that the new CNVS is a valid score that can be used for either clinical or research settings. Future investigations should include the ability of the CNVS to assess change in puppies over time and to predict short and long term survival and morbidity.

Keywords: Neonatal vitality score, puppy, scoring system, resuscitation

Comparing deslorelin implants or oral diethylstilbestrol to induce estrus in the bitch

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Estrus induction is sometimes requested when prolonged interestrus intervals have occurred, or to produce more embryos in a shorter time period. The objective of the study was to determine the efficacy of estrus induction in bitches using deslorelin implants (OVUP) or oral diethylstilbestrol (DES) in an embryo transfer project. The hypothesis was that DES would be as efficacious at inducing estrus as OVUP. Estrus induction was attempted 29 times in 19 adult, Walker-type hound (~30 kg) bitches housed at the Louisiana State University School of Veterinary Medicine. The true interestrus was not always known before the initial induction protocol, and if unknown, was calculated based on the date of a progesterone concentration of $\leq$ 2ng/mL after enrollment in the study. Subsequent cycles had interestrus intervals calculated from the previous day one of cytologic diestrus. All bitches had estrous cycles monitored by vaginal cytology every other day until day one of cytologic diestrus and serum progesterone until a value confirming ovulation (>5 ng/mL). Oviductal embryo recovery following ovariectomy at 10 d post-LH peak (progesterone rise > 2 ng/mL) was attempted in nine bitches following estrus induction using 5 mg oral DES daily for 5 to 7 d (n=5; OVX-DES), or a single subcutaneous deslorelin implant (Ovuplant®, Wyeth Animal Health, Guelph, Ontario, CA) inserted into the vulvar mucosa (n=4; OVX-OVUP). Non-surgical or surgical embryo recovery 13 to 15 d after an initial rise in progesterone $\geq$ 2 ng/mL was attempted following estrus induction using OVUP (n=10; OVUP-1) followed by a second attempt (n=8; OVUP-2); and a single bitch had four attempts (OVUP-3). Results for each group were compared to the OVX-DES group by Fisher's exact tests.

Corpora lutea were found after ovariectomy in two of five bitches in the OVX-DES group, however progesterone only increased to 3 ng/mL after day one of cytologic diestrus. Because of this abnormal progesterone pattern, these cycles were not considered normal. The interestrus intervals, estrual response rates (P value), and days to LH peak from treatment were 22.2 $\pm$ 9.0, 0% (0/5), 26.5 $\pm$ 13.4 for the OVX-DES group, 24.8 $\pm$ 8.8, 100% (4/4) (P=.0079), 8.8 $\pm$ 0.5, for the OVX-OVUP group, 68.2 $\pm$ 29.4, 60% (6/10) (P=0.044), 11.4 $\pm$ 0.8, for the OVUP-1 group, and 22.2 $\pm$ 9.0, 100% (8/8) (P=0.0008) 11.6 $\pm$ 2.9 for the OVUP-2, group, respectively. No embryos were recovered from either of the OVX groups, and embryos (n=1) or pregnancies (n=4) were found in 33.3% (5/15) of the successful deslorelin estrus inductions.

The hypothesis that DES is as efficacious at inducing estrus as OVUP was not supported. The OVUP treatment was more efficacious than DES.

Keywords: Estrus, estrous induction, deslorelin, canine, diethylstilbestrol

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Canine pulmonary surfactant synthesis during late fetal development

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Introduction

Histological lung maturation comprises five phases of development: embryonary, pseudoglandular, canalicular, saccular and alveolar. The canalicular phase is characterized by the development of the type I and type II pneumocytes, responsible for surfactant synthesis.[1] The major function of surfactant SP-B protein is to reduce alveolar surface tension and thus, prevent lung collapse at the end of the expiratory phase. Impairment in SP-B production renders the newborn unsuitable for extra-uterine respiration. Therefore, the extent of pulmonary surfactant production can certainly be correlated with fetal maturity. This study aimed to determine the gestational period from which the onset of surfactant SP-B production begins in canine fetuses.

Material and methods

A total of 13 fetuses of different breeds were utilized. Animals were allocated into three groups, according to gestational period: 45 to 49 days of gestation (n = five), 50 to 54 days of gestation (n = five) and 55 to 63 days of gestation (n = three). After fetal euthanasia, fetuses were submitted to pulmonary lobectomy. The fetal lungs were washed plentifully with saline solution and then, samples were removed from each lobe. Lung lobes were cut into 2 cm^3fragments and stored in 10% formalin at room temperature until processing. The lung fragments were embedded in paraffin, cut into 5 μm cross sections and subjected to immunohistochemistry reaction with primary specific antibody for surfactant protein SP-B (Chemicon International, Temecula, CA) and a biotinylated secondary antibody (DakoCytomation, Carpinteria, CA). The sections were counterstained and evaluated under optical microscopy (10-100X) for a positive immunostaining at pneumocytes type II cells.

Results and discussion

Surfactant SP-B protein was identified in pneumocytes type II cytoplasms from all fetuses with at least 50 days of gestational age. In canine fetuses, the canalicular phase of lung development corresponds to the interval between the 48th and 57th days of pregnancy.[1] Thus, it is possible to infer that the initial SP-B expression is similar among dogs and humans. However, respiratory system maturation depends on a successful process of morphogenesis and angiogenesis, adequate surfactant production and composition and proper fetal adaptation to stress. Our results will allow for the accurate indication of hormonal therapy for the stimulation of fetal maturation. Since previous studies concerning fetal lung development and surfactant production in canine species are limited and scarce, future investigations are needed to broaden our understanding in this area.

Keywords: Surfactant; SP-B protein; immunohistochemistry; lung development, dog

Reference

1. Sipriani TM, Grandi F, Silva LCG, et al: Pulmonary maturation in canine foetuses from early pregnancy to parturition. Reprod Domest Anim 2009; 44:137-140.

LH testing is accurate for diagnosing the presence or absence of testicular tissue in dogs

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Introduction

The rapid immunomigration assay (WITNESS® LH, Synbiotics, Kansas City, MO) test kit provides an accurate, semi-quantitative measurement of canine and feline luteinizing hormone (LH). Although originally designed as a canine ovulation timing aid, the WITNESS® LH test kit is commonly used for diagnosing the presence or absence of retained ovarian tissue in bitches and queens. In ovariectomized (spayed) females, LH concentrations remain persistently elevated due to the lack of negative feedback from the gonads. Therefore, we hypothesized that determining LH concentrations could also be used to diagnose the presence or absence of testicular tissue in male dogs. The objective of this study was to compare results from the WITNESS® LH test kit between intact and castrated male dogs.

Materials and methods

Six purebred dogs and three crossbred dogs were represented with body weights ranging from 17-70 lbs. Venous blood samples were collected from intact (n=6) and castrated (n=3) post-pubertal male dogs. The blood was allowed to clot and serum was separated by centrifugation. All of the tests were conducted following the manufacturer's instructions. Using the pipette provided, 3 drops of serum were added to the serum well of the test device. The test device was kept horizontal and undisturbed for twenty minutes when results were read. A positive result occurred when a line appears in the test area which is of greater intensity than the control line. When this occurs, the LH concentration is >1 ng/mL.

Results

Samples collected from all of the intact male dogs were ≤1 ng/mL (negative) whereas samples collected from all of the castrated male dogs were >1 ng/mL (positive) (Table 1).

Discussion

LH testing using the WITNESS® LH test kit is an accurate method for diagnosing the presence or absence of testicular tissue in dogs. Additional testing in known cryptorchid male dogs is needed to confirm efficacy for diagnosing this condition.

Table 1. Results from the WITNESS® LH test kit in intact and castrated male dogs.

Reproductive Status	Breed	Result
Intact	Springer spaniel	Negative
Intact	Dachshund	Negative
Intact	Doberman	Negative
Intact	Mix	Negative
Intact	Mix	Negative
Intact	Doberman	Negative
Intact	Scottish terrier	Negative
Castrated	Doberman	Positive
Castrated	Jack Russell terrier	Positive
Castrated	Mix	Positive

Keywords: Castration, cryptorchid, dog, luteinizing hormone, rapid immunomigration assay

Somatic cell nuclear transfer-derived calves: can we predict neonatal viability?

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In order to identify perinatal parameters that will allow the detection of high-risk calves and predict neonatal outcome, 18 commercial somatic cell nuclear transfer (SCNT)-derived pregnancies were evaluated prospectively. Our hypothesis was that pre-partum ultrasonographic parameters (mean, maximum and minimum fetal heart rates, fetal fluid and movement scores), gestation length, method of delivery, gender and birth weight would allow prediction of neonatal outcome. Calves were delivered vaginally 4.7 ± 1.5 d (n=3) or via Cesarean section (n=15) 2.4 ± 5.9 d from expected calving date. Mean, maximum, and minimum fetal heart rates, fetal movement (score 0 to 3) and fetal fluid echogenicity (score 0 to 3) were evaluated via transabdominal ultrasonography twice daily starting 3.8 ± 5.8 d before expected calving date. Data were analyzed using SAS 9.1 for Windows Software, and Student's t test, regression analysis and Fisher's exact test were performed where appropriate. Data are expressed as mean ± SD. Calf survival rate was 61% (vaginal delivery 100 %, Cesarean section 53 %, p=0.2451). Survival rate of calves delivered via Cesarean-section with prior administration of dexamethasone was 58 % (7/12) and 33.3 % (1/3) without dexamethasone (p=0.5692). Fewer males (5/12, 41.7 %) survived than females (6/6, 100 %) (p=0.0377). Non-surviving calves (-1.3 ± 6.7 d) were delivered earlier regarding expected calving date than surviving calves (5.5 ± 2.8 d) (p=0.0356). Non-surviving calves (63.1 ± 9.2 kg) were heavier than survivors (59.3 ± 9.7 kg) (p=0.0134). Weight was not correlated with gestation length (p=0.3556). Mean fetal heart rate in surviving calves was 105.8 ± 16.1 bpm (minimum value observed 81 bpm, maximum value observed 174 bpm), and in non-surviving calves, 116.5 ± 15.9 bpm (minimum value observed 92 bpm, maximum value observed 192 bpm). Mean (p=0.0069), maximum (p=0.0005) and minimum fetal heart rates (p=<.0001) were higher in non-surviving calves. A mean (p=0.0128) or minimum (p=0.0429) fetal heart rate >125 bpm in one or more examinations was associated with neonatal death. When the minimum or mean fetal heart rate remained ≤125 bpm, 73.3 % and 83.3 % of calves lived, respectively. A maximum fetal heart rate >125 bpm was associated with fetal death only when fetal fluids were ≥1 during the same examination. Fluid and movement scores were not associated with neonatal outcome. Therefore, calf gender, gestation length, birth weight and fetal heart rates seem associated with neonatal outcome in bovine SCNT-derived pregnancies and can help identify at risk calves perinatally.

Keywords: Bovine, somatic cell nuclear transfer, pregnancy, ultrasound, monitoring

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Treatment of clinical endometritis with prostaglandins: a clinical trial in a commercial dairy farm

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The objective of this study was to assess the efficacy of prostaglandin $F_{2\alpha}$, to treat clinical endometritis (CE) in dairy cows under commercial conditions. A longitudinal study was conducted in one dairy farm in Argentina which housed 6326 Holstein cows (1 lactation, n=2053; ≥2 lactation, n=4273) that calved from January 2007 through December 2008. Each postpartum cow was examined for diagnosis of clinical endometritis (CE) once between 15 and 45 days postpartum (dpp) at a monthly herd visit. At examination (EX1), cows were first inspected for presence of fresh and/or dry discharge on the vulva, perineum, or tail; and then the mucus content of the vagina was evaluated for color, proportion of pus to mucus, and odor; and a score was assigned as follows: clear mucus (CE0), predominantly clear with flecks of pus (CE1), purulent but not foul-smelling (CE2), or purulent or red-brown and foul smelling (CE3). Cows diagnosed CE0, were not treated; cows diagnosed with CE1 and CE2 were randomly assigned to one of two treatments (placebo, PLA; PGF, cloprostenol, 0.150 mg i.m., Ciclase®, Syntex SA, Buenos Aires, Argentina); and cows with CE3 were treated with an antibiotic (oxytetracycline, 12 g, i.m., Terramicina LA®, Pfizer SA, Buenos Aires, Argentina) and PGF. All cows were re-examined (EX2) following the same criteria at the next monthly visit (30 d) to determine the outcome of treatment. The median interval from calving to EX1was 30.5±0.1 d, to EX2 55.4±0.2 d, and between examinations was 30.0±0.1 d. First lactation cows had higher prevalence of CE compared to 2+ lactation cows (37% vs.24%, P<0.01). Significant differences in prevalence of CE were found between seasons (summer [32%], fall and spring [28%], winter [25%]); P<00.1) and between years (2007 [27%], 2008 [30%]; P<0.01). Cows with abnormal calvings had 16% more CE compared to cows with normal calvings (42% vs. 26%; P<0.01). The shorter the interval from calving to EX1, the higher the prevalence of CE (14-20 d, 58%; 21-27 d, 37%; 28-34 d, 23%; 35-41 d, 17%; 42-48 d, 15%; 49-55 d, 17%; P<0.01). At EX1, the prevalence of CE0 was 72%, of CE1 14%, of CE2 10%, and of CE3 4%. At EX2, 75% of cows with CE1, 62% of cows with CE2 and 59% of cows with CE3 were cured (CE0). Cows in PLA and PGF had the same cure rate for CE1 (74% [294/396] vs. 76% [183/240]; P>0.10) and for CE2 (61% [231/379] vs. 64% [179/279]; P>0.10). Cows with CE1 had a higher cure rate compared to cows with CE2 (75% [477/636] vs. 62% [410/658]; P<0.01). In conclusion, first lactation cows, cows with abnormal calvings, cows with summer puerperium, and cows with shorter interval from parturition to EX1 had higher prevalence of CE. Cows with CE1 and CE2 had a 75% and 62% cure rate after treatment with PGF, and cows with CE3 had a 59% cure rate after treatment with ATB and PGF. The use of PGF was not effective to improve the cure rate in CE1 and CE2.

Keywords: Clinical endometritis, postpartum examination, prostaglandins, cure rate

Cryopreservation and fertility of Bighorn (*Ovis canadensis c.*) cauda epididymis semen

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Bighorn sheep (*Ovis canadensis canadensis*) have high morbidity and mortality rates due to *Pasteurella/Manhemia spp.* pneumonia when they come into contact with domestic sheep (*Ovis aries*). We have reported previously the production of hybrid pregnancies between the two species using laparoscopic insemination with fresh semen.[1] The objective of the present experiment was to determine the fresh and post-thaw quality and fertility of cauda epididymis semen collected postmortem from Bighorn rams that were suffering from pneumonia.

Four Bighorn rams were exposed to domestic sheep for a period of 30 days as part of a study on pneumonia. Rams were euthanized immediately if severely ill or upon completion of the study. The testicles were collected within the scrotal sac and processed within six hours of death. The cauda epididymis was dissected and semen was harvested by float-up technique following mincing. A commercial ovine freezing extender (IMV Technology, St Paul, MN) with 20% egg yolk was used for semen harvesting and freezing. Semen was diluted to provide approximately 100 million spermatozoa per mL then cooled slowly to 5 °C and equilibrated for 3h at this temperature before freezing. Semen was frozen in 0.5 mL French straws placed 4 cm above liquid nitrogen for 20 min then plunged in liquid nitrogen. Morphology was evaluated after 10 min of incubation at room temperature following collection. Progressive motility was evaluated immediately before cooling and after thawing (37 °C for 30 sec). To evaluate fertility frozen-thawed samples were used to laparoscopically inseminate (LAI) eight estrous synchronized ewe-lambs as described previously.[1] Pregnancy diagnosis was performed by ultrasonography at 30 days.

All samples obtained showed very high progressive motility (>80%) and high normal morphology (>90%). There was no significant difference in semen quality amongst rams ($P<0.05$) despite the fact that some had had a fever of 40.5 °C (105 °F) for up to five days before euthanasia. The mean (±SEM) percent post-thaw progressive motility was 63.7 ± 1.8. Three ewes became pregnant following LAI. Two lambed twins after 155 and 156 days gestation, respectively. The third ewe lambed triplets at 156 days. Lamb weight varied from 1.9 to 3.4 kg. These results show that Bighorn cauda epididymis semen maintain fertility after cryopreservation. This technique could be used to preserve genetic diversity in Bighorn flocks if the testes are collected from sick or dying wild males in a timely manner.

Keywords: Genetic preservation, insemination, sheep, laparoscopy, hybrids

Reference

1. Rodriguez JS, Tibary A, Sandoval S, et al. Production of hybrid (Bighorn x domestic sheep) lambs by laparoscopic artificial insemination using Bighorn fresh semen collected by electroejaculation. Clin Therio 2009; 1:243.

Quantitative morphometric analysis of the utero-placental vascular network and angiogenic effects of tocopherols in late pregnant ewes

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Objective

To compare angiogenic morphometric parameters of the utero-placental vascular network of late pregnant ewes supplemented with tocopherol or placebo.

Design

Clinical trial.

Subject

18 late pregnant ewes, crossbred; 2 to 6 years of age.

Interventions

Oral supplementation of 500 mg of alpha tocopherol (aT; N=6) or 1000 mg of gamma tocopherol (gT; N=7) or placebo (CON; N=5).

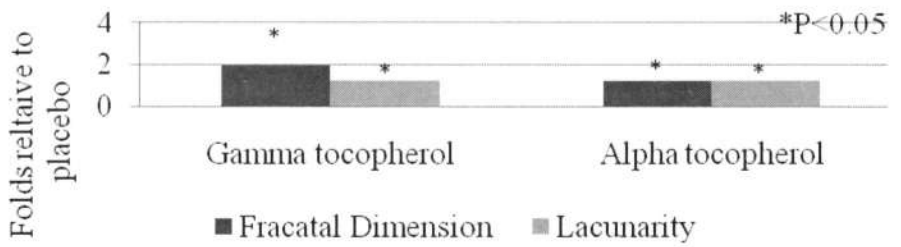
Methods

Ewes were supplemented daily from 100 to 137 days of gestation. At the end of the supplementation, all ewes were euthanized and placentomes near the umbilicus were collected. Uterine and placentomal tocopherol concentrations were estimated by HPLC procedure. Placentomal sections were stained with hematoxylin and eosin for morphometric evaluation. Image processing and analysis were performed using ImageJ 1.42q (NIH, USA) to evaluate the fractal dimension and lacunarity. Kruskall-Wallis analysis was used to compare the differences between treatment groups.

Results

The median aT concentration (mg/kg) in placentome (9.19) and uterus (5.23) was greater in ewes supplemented with aT compared to ewes in gT and CON groups ($P<0.05$) The median gT concentrations (μg/kg) in placentome (607) and uterus (737.7) were greater in ewes supplemented with gT compared to ewes in the aT and CON groups ($P<0.05$). Increased fractal dimension and decreased lacunarity were observed in tocopherol treated ewes compared to placebo treated ewes (Figure 1; $P<0.05$).

Figure 1: Angiogenic morphometric parameters of the uteroplacental vascular network of late pregnant ewes supplemented with tocopherol or placebo



Conclusion

The placentomal morphometric analysis of late pregnant ewes supplemented with aT or gT showed an increase in angiogenic parameters in utero-placental vascular units.

Keywords: Sheep, vitamin E, feto-maternal unit, fractal dimension, lacunarity

Early apoptosis in follicular versus ovulated oocytes in superovulated goats

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Our laboratory has found that while approximately 80% of oocytes aspirated from slaughterhouse bovine ovaries take up a vital stain indicating early apoptosis, nearly 100 % of oocytes obtained from oviductal flushes in superovulated goats take up this stain. The objective of this study was to investigate whether these differences were related to species, or to differences in aspirated follicular oocytes versus ovulated oocytes flushed from the oviduct.

Ten alpine/Saanen female goats, scheduled for euthanasia were divided into two groups: Group A, preovulatory, aspirated follicles; Group B, ovulated, flushed oocytes and aspirated follicles. A short-progesterone protocol was used to induce estrus synchronization and superovulation. Briefy, this protocol included, a small ruminant CIDR (Eazi-Breed™ CIDR-G®, DEC International, Hamilton, NZ) (insertion Day 0, removal Day 3), two 5 mg doses of $PGF_{2\alpha}$ (Lutalyse®, Pfizer Animal Health, New York, NY) (Days 0, 3), 256 mg of FSH (Folltropin-V®, Bioniche Animal Health, Belleville, ON, CA) in 8 equal doses (Day 1 to Day 4), and 5 μg GnRH (Cystorelin®, Merial Canada Inc, QB, CA) (Day 5). Group A was terminated on Day 5 (1 ml/10 lbs, Beuthanasia-D, Intervet/Schering-Plough Animal Health, Millsboro DE), and Group B animals were terminated on the morning of Day 6. One animal in Group A did not respond to the protocol. In both groups, oocytes were aspirated from all follicles. For Group B, oocytes were flushed from the oviduct with 20 ml of embryo collection medium (Biolife™ Advantage Complete Flush Medium, AgTech, Manhatten, KS). All oocytes were stained with Yo Pro-1 and propidium iodide (Invitrogen, Carlsbad, CA) to detect apoptotic and dead oocytes respectively, and evaluated using a Zeiss Axiovert 200 fluorescent microscope with Axiovision software. Data were analyzed using a two-tailed Fisher Exact Probability Test.

The results are seen below:

	Total	Apoptotic (%)	No Stain (%)	Questionable (%)
Group A: Preovulatory, aspirated	24	14 (58.3)[a]	5 (20.8)[a]	5 (20.8)
Group B: Ovulated, aspirated	22	13 (59.1)[a]	5 (22.7)[a]	4 (18.2)
Group B: Ovulated, flushed	43	42 (97.7)[b]	1 (2.3)[b]	0

[a,b] different superscripts within columns are significantly different, $P<0.01$.

In conclusion, significantly more ovulated oocytes took up the stain for early apoptosis compared to oocytes aspirated from follicles on either preovulatory or ovulatory ovaries. The aspiration results are similar to those seen in aspirated bovine oocytes. Further work is needed to determine whether the uptake of stain seen in ovulated oocytes is truly early apoptosis versus membrane changes related to ovulation or superovulation. This is important, as we have produced two live goats from early embryos that took up the vital stain for apoptosis.

Keywords: Oocytes, goats, apoptosis, superovulation, fluorescent stains

Effect of Excenel RTU® and Polyflex® on cure and reproductive performance of dairy cows diagnosed with acute puerperal metritis

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The objective of the study was to evaluate the effect of Excenel RTU® and Polyflex® for acute puerperal metritis on cure and reproductive performance in dairy cows as compared to non-metritic control cows. Cows were monitored daily for 10 days from day 1 post-calving. Cows (N=500) with rectal temperature >39.4 °C (<103.0 °F) and atonic uterus, malodorous, watery vaginal or uterine discharge were classified as metritic cows and were randomly allocated to one of two treatment groups. Cows in CEF group (N=251) received 2.2 mg/kg BW of ceftiofur hydrochloride (Excenel RTU®, Pfizer Animal Health, New York, NY) IM, once daily for 5 days. Cows in AMP group (N=249) received 2.3 mg/kg BW of ampicillin sodium (Polyflex®, Boehringer Ingelheim Pharm Inc, Ridgefield, CT) IM, once daily for 5 days. All cows received supportive therapy and any cow whose condition was life threatening was deemed a treatment failure and given alternative treatment. Cows (CON; N=470) with no signs of metritis and rectal temperature >39.4 °C were classified as non-metritc control for comparison. These cows were matched to treated cows by DIM and parity. The outcomes measured were cure at 10 days post-treatment (metritis Y/N) and cure at 33 days post-treatment (uterus and cervix size, and presence or absence of discharge). Cows were monitored through the completion of the lactation, or until 525 days post-calving. PROC LOGISTIC was used to model the cumulative probability of cure at 10 and 33 days and PROC PHREG of SAS was used to determine the daily pregnancy risk and time to first service for treated and control groups.

There was no difference in cure at 10 days and cure at 33 days between CEF and AMP ($P>0.05$) and no difference in daily pregnancy risk and first service risk between CEF and AMP treatment groups ($P>0.05$). The daily pregnancy risk was reduced 23% for cows treated with ceftiofur and 28% for cows treated with ampicillin compared to CON group ($P<0.01$). The first service risk was reduced by 23% for CEF group and by 28% for AMP group compared to CON group ($P<0.01$). The service per conception was 3.3, 3.7 and 3.7 for CON, CEF and AMP groups, respectively. In conclusion, dairy cows diagnosed with acute puerperal metritis that received either Excenel RTU® or Polyflex® IM once daily for 5 days showed no difference in cure, and showed similar reproductive performances. However their reproductive performance was reduced compared to their cohort of non-metritic control cows.

Keywords: Dairy cattle, uterine disease, parenteral antibiotic, cure, reproductive performance

Pregnancy rates in dairy cattle inseminated with different numbers of progressively motile sperm

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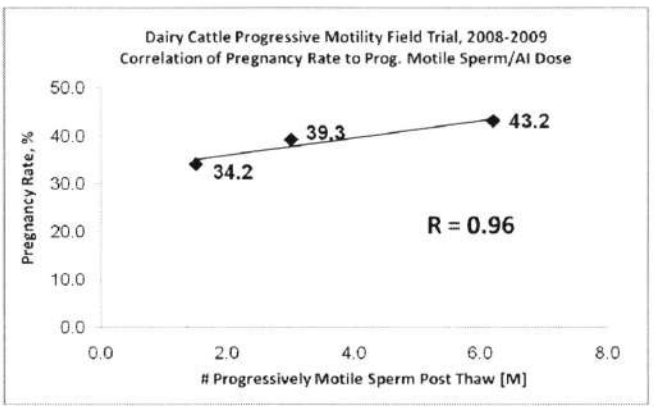
The objective of this field trial, conducted in a commercial setting, was to evaluate the impact on pregnancy rates of dairy cattle which were artificially inseminated with straws dosed with varying amounts of progressively motile post-thaw (PMPT) sperm.

The trial was conducted in two phases. In phase 1, a post-thaw progressive motility loss factor (PMLF), representing the percent decrease of progressively motile cells associated with freeze/thawing, was determined for each of the four bulls used in the trial. This phase included both testing the accuracy of preparing straws for artificial insemination (AI) based on estimated PMPT sperm and also preparing the straws with different estimated PMPT sperm numbers as stated below. In phase 2, pregnancy rates in dairy cattle inseminated with straws containing different estimated PMPT sperm numbers were compared. Both fresh and post-thaw frozen semen were evaluated using an automated sperm quality analyzer for bulls (SQA-Vb). AI doses were prepared using B-Sperm™ dosing software (Medical Electronic Systems/A-TECH, Israel). Once the PMLF was established, ten ejaculates from each bull were collected. Each ejaculate was split into four groups (A, B,C, D) and straws were prepared. Three groups of straws (A, B, C) were targeted to contain 1.5, 3.5 and 7.0 million PMPT sperm/straw respectively and the study control group (D) was targeted to contain 15.0 million total sperm/straw, representing the standard procedure at the collaborating bull stud (SION AI Company, Israel). MedCalc software (MedCalc Software, Mariakerke, Belgium) was used for statistical evaluation of the trial results.

Seventy-six post-thaw AI straws from each group were tested for quality control purposes using the SQA-Vb. In groups A, B and C, the number of PMPT sperm was tested. The mean (+/- SE) values for PMPT sperm were: (A) 1.5 (+/- 0.05); (B) 3.0 (+/- 0.09) and (C) 6.2 (+/- 0.18) million progressively motile spermatozoa. The mean (+/- SE) value for total sperm per dose in group D (Control) was 13.6 (+/- 0.15) million. As these results were considered satisfactory for trial purposes, a total of approximately 8,000 AI doses (2,000 per group) were then prepared and frozen by stud technicians. In phase 2 of the trial 6,494 Holstein cows on more than 500 farms were artificially inseminated with straws from group A, B, C or D between January and April 2009. Pregnancy testing by per-rectal palpation was conducted by veterinarians at approximately 42 days post-insemination. Both insemination and pregnancy testing were performed blindly. Pregnancy rates (per cow per cycle) were: Group A 34.2%; Group B 39.3%; Group C 43.2%; Group D 38.6%.

Significant differences occurred between groups A (1.5 M Prog Mot) and D (15 M Total), C (7.0 M Prog Mot) and D (15 M Total), and A (1.5 M Prog Mot) and C (7.0 M Prog Mot). The relative differences in pregnancy rates in the experimental groups A, B and C vs. the control were: -11.4%, 1.8% and 11.9%, respectively. Pregnancy rates in groups A, B and C were correlated with numbers of progressively motile spermatozoa per AI dose ($r = 0.96$).



It is concluded that the number of progressively motile sperm per AI dose is strongly related to subsequent pregnancy rates. Further, that it is possible to accurately produce AI straws of frozen bovine semen based on targeted post thaw numbers of progressively motile sperm in a commercial setting. Use of these findings could help improve bovine AI reproductive performance while allowing more effective utilization of superior bulls.

Keywords: Sperm progressive motility, artificial insemination, frozen bovine semen, SQA-Vb, PMLF

Association of adiponectin, testosterone, prolactin and sperm DNA fragmentation index in Holstein bulls

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Adiponectin and prolactin are pleiotropic regulators of a large set of biological functions including gonadal steroidogenesis and thermoregulation. This study was undertaken to determine the effect of serum adiponectin, testosterone, and prolactin on sperm DNA fragmentation index (DFI). Blood samples from ten yearling bulls were collected bi-weekly by jugular venipuncture using serum separator evacuated tubes using18-gauge needles (Becton Dickinson and Co., Franklin Lakes, NJ) between 0800 and 1000 h on d 0, 14, 28, 32, 46, 60, 72, 84, 96, and 108 of the study to assess serum testosterone, prolactin and adiponectin concentrations. On the same days and intervals, semen samples were collected by artificial vagina and were frozen in 0.5 mL straws (20×10^6/mL) using egg-yolk glycerol extender. Testosterone concentrations were determined using a total testosterone RIA. Prolactin was determined using a previously validated assay.[1] Adiponectin concentrations were determined by ELISA. The frozen semen samples were thawed at 35 °C and subjected to flow cytometry analysis of sperm chromatin structure to estimate the DFI. Previously we reported that no differences were observed in DFI for bulls between sampling days ($P > 0.1$) but differences were observed between bulls within sampling days ($P < 0.001$).[2] Hence, the DFI and hormone concentrations between sampling days were averaged for analysis. Adiponectin concentration ranged from 178 to 654 ηg/mL. Sperm DFI ranged from 1.03 to 4.12. Testosterone ranged from 0.5 to 11.5 ηg/ mL. Prolactin ranged from 2.17 to 113.7 ηg/mL. Correlations between the DFI and hormones were determined using Pearson's correlation coefficient. Adiponectin was correlated negatively to testosterone ($r = -0.81$) and positively to DFI ($r=0.78$) and prolactin ($r=0.74$) ($P<0.05$). Prolactin was correlated positively to DFI ($r=0.89$) and negatively to testosterone ($r=-0.81$) ($P<0.001$). In conclusion, adiponectin influences sperm DFI possibly via gonadal steroidogenesis by altering testosterone concentration and via thermoregulation by altering prolactin. However, the prolactin role needs to be studied further as it positively influences the sperm DFI.

Keywords: Bull, sperm DFI, adiponectin, prolactin, testosterone

References

1. Miller AR, Stanisiewski EP, Erdman RA, et al: Effects of long daily photoperiod and bovine somatotropin on milk yield in cows. J Dairy Sci 1999;82:1716-1722.
2. Kasimanickam R, Eyestone WH, DeJarnette MJ, et al: An estimate of the normal variation in the sperm DNA fragmentation index of Holstein bulls, and its association with serum testosterone and prolactin, over two spermatogenic cycles [abstract]. Theriogenology 2008;70:584.

Effect of side of embryo transfer in relationship to location of the corpus luteum on pregnancy rate in camels (*Camelus dromedarius*)

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Embryo migration and establishment of pregnancy in the left uterine horn regardless of the ovarian side where the corpus luteum is located is an intriguing aspect of camelid reproduction. Investigation of pregnancy rate after transfer to the same side or opposite side to the CL-bearing ovary offers a good method for the study of early maternal recognition signaling. In the present study we hypothesized that transfer to the horn ipsilateral to the CL-bearing ovary would result in significantly higher pregnancy rates than transfer to opposite side.

During three consecutive years, embryos were transferred to recipients either ipsilateral or contralateral to side of ovulation. Hatched blastocysts were collected non-surgically eight days after mating and transferred non-surgically to recipients that had ovulated 2 days before the donor. All transferred embryos were classified morphologically as excellent. Data from recipients that had double ovulations were excluded. Pregnancy status was determined by transrectal ultrasonography at 18 days and confirmed at 90 days. Pregnancy rates were analyzed using multiple comparison procedures for contingency table. Results are shown in Table 1. There was a significant effect of the year ($P<0.05$). Ipsilateral transfer when the CL was on the right ovary resulted in the highest pregnancy rate. The pregnancy rates obtained by transfer to the right uterine horn when the CL was on the left were unexpected.

Table 1: Pregnancy rate (PR %) obtained following transfer of embryo to the horn ipsilateral or opposite horn to the CL bearing ovary

CL side /Horn side	Right/Right	Left/Left	Right/Left	Left/Right
Year 1 PR (n)	69.6a (102)	47.8b (90)	53.3b (30)	50.0b (28)
Year 2 PR (n)	61.5a (39)	57.1a (21)	52.6a (78)	47.4b (97)
Year 3 PR (n)	68.6a,b (70)	71.8a,b (32)	57.1a (28)	72.6b (62)
Total PR (n)	67.8a (211)	54.5b (143)	53.7b (136)	56.1b (187)

Different letters within the same row represent significant difference ($P<0.05$)

These results suggest that the asynchrony (2 days) between the donor and recipients provides better results without regard on which side the embryos are transferred. A study is being conducted to look at the same parameters following transfer to synchronous recipients.

Keywords: Embryo, pregnancy recognition, luteolysis

Effect of volume and timing of induction of ovulation on conception rate following deep horn insemination in camels (*Camelus dromedarius*)

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Artificial insemination (AI) in camelids offers many challenges due to the induced nature of ovulation in this species as well as the viscous nature of semen. In the camel racing industry, very few males are kept intact and therefore availability of superior males with known track records in racing is limited. In this study we investigated the feasibility of fresh semen insemination with low sperm numbers using deep horn insemination (DHAI). Our objectives were to compare two volumes of inseminate and timing of ovulation induction treatment in relationship to AI.

Multiparous female camels that have passed the breeding soundness examination required in our laboratory were allocated to 4 groups. Group 1 (n=25) and 2 (n=24) were inseminated with 0.25 mL extended fresh semen. Groups 3 (n=25) and 4 (n=24) were inseminated with 0.5 mL of extended fresh semen. Ovulation was induced with buserelein (25 μ, IM) at the time of insemination (Groups 1 and 3) or 24 hours prior to insemination (Groups 2 and 4). Semen was collected by a modified bovine artificial vagina from one male and diluted with a commercial extender (Green Buffer®, IMV Technologies, L'Aigle, France) with added 20% egg yolk. All females were inseminated with a flexible catheter guided transrectaly to the tip of the uterine horn ipsilateral to the ovary with a dominant follicle (minimum 12 mm and maximum 15 mm). Concentration of inseminates was adjusted to contain approximately 24 million progressively motile spermatozoa. Ovulation was confirmed by serum progesterone level 7 days after insemination using an ELISA kit (Merieux, France). Pregnancy diagnosis was performed by transrectal ultrasonography at 18 days following DHAI. Pregnancy rates were compared using Chi-square analysis.

Pregnancy rates for group 1 to 4 were respectively, 0 (0/25), 8.3% (2/22), 48% (12/25) and 58.3% (14/24). There was a significant effect of insemination volume ($P<0.001$) on pregnancy rate. Although the pregnancy rate in camels induced to ovulate at the time of insemination was lower than that of camels inseminated 24 hours following GnRH, this difference was not statistically significant.

These results show that acceptable pregnancy rates can be achieved with low sperm number (24 million vs the usual dose of 100 to 150 million) when DHAI is used. This technique could solve the problem of shortage of semen from highly valuable males during the peak of the breeding season. Timing of ovulation induction does not seem to be an important factor when fresh semen is used. However, other studies in progress in our laboratory show an advantage in administering GnRH 24h before insemination particularly when reducing the dose to 10 million spermatozoa or when using frozen-thawed semen.

Keywords: Dose, fertility, camelid, ovulation, induction

Effect of the testicular size of the sire group on the pregnancy rate in alpacas (*Vicugna pacos*)

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The positive correlation between size and weight of the paired testicles and fertility is well established in all traditional livestock species. Alpacas are distinct in that the ratio between body and testicular weight is very low (0.04) compared to that observed in bulls (0.18) and rams (1.40).[1] We have observed a large variation in testicular size of adult breeding alpacas used in the Peruvian Altiplano herds. We hypothesized that in the group mating system practiced in this area, pregnancy and birth rate will be affected by the mean testicular size of sires in mating groups. Our objective was to determine if females joined to males with smaller testicular size will achieve lower overall birthing rates than females joined with larger testicular size males.

Eighty (80) parous healthy females were randomly assigned to one of two mating groups. Group 1 (n=40) was joined to 4 adult males with large testicles with a mean testicular length and width of 4.8 ± 0.25 cm and 3.6 ± 0.16 cm, respectively. Group 2 (n=40) was joined to 4 adult males with small testicles with a mean testicular length and width of 3.0 ± 0.08 cm and 1.8 ± 0.5 cm, respectively. The mean testicular size was significantly different ($P<0.05$) between the male groups. The breeding trial was conducted at the end of the traditional breeding season over a 42 day period at the Sumac Tarpuy Station, Ayaviri, Puno, Peru, at 3,900 meters above sea level. Both groups of females were managed similarly following the breeding season. Parturition rates were recorded during the birthing season for both groups and compared using chi-square analysis.

The parturition rate in Group 1 and Group 2 were 47.5% and 30%, respectively. Although females mated to large testicular sized males had a higher odd ratio to become pregnant, this difference was not statistically different ($P=0.108$). The overall low fertility observed in this trial (38.8%) and the relatively small number of females used may be a reason for this lack of statistical significance. Fertility tended to be higher when female alpacas were bred to males with normal sized testicles which justifies considering this trait in selecting future sires. Further experiments are planned to determine the effect of other factors such as the female to male ratio and length of the breeding season on the performance of males with different testicular sizes.

Keywords: Alpaca, fertility, conception, reproductive efficiency

Reference

1. Setchell BP: The mammalian testis. Ithaca (NY); Cornell Universtity Press; 1978.

Uterine torsion in late gestation alpacas and llamas: 60 cases (2000-2009)

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Reproductive emergencies in late gestation camelids are dominated by uterine torsion. This retrospective study was performed to evaluate clinical treatment and outcome, and to attempt to identify risk factors such as time to referral, laboratory abnormalities, parity, and gestational age. Survival of dams and crias was also calculated and compared to treatment modalities.

The cases included alpacas (n=56) and llamas (n=4). Mean (± SD) age of females was 5.88 ± 2.88 years (range 2-13). Maiden females represented 21.7% of animals, and 78.3% were multiparous. The mean gestational age at the time of presentation was 332.78 ± 26.3 days (range 246-376). Average time to referral was 24.9 ± 28.5 hours (range 0-158). Methods of correction included 3 treatment groups: rolling only (ROL) (60%, n=36), cesarean section only (CS) (23.3%, n=14), and rolling followed by cesarean section (RCS) (16.7%, n=10). There was no significant difference between mean gestational length and treatment method implemented (p=0.11).

The direction of the torsion was clockwise in 81.7% of cases and counterclockwise in 18.3% of the cases. Severity of torsion was categorized as 90° (14.3%), 180° (26.3%), 270° (10.7%), or 360° (48.2%). There was a significant effect of degree of torsion on method of correction, with 360° torsions more likely to undergo CS, either as a solitary treatment method or after rolling ($p<0.05$).

Survival data were available for all females (96.7%, n=58). Two females died, both after CS. Cria survival following correction was 78.3% and was significantly higher after ROL (100%, n=36) than following CS (71.4%, n=10) or RCS (70%, n=7) ($p<0.05$).

Laboratory assessment by complete blood count and serum chemistry were within established reference ranges in 65% of cases for which data were available. The most common abnormalities were toxemia (19.9%), hypocalcemia (< 9.0 mg/dL) (5%), toxemia plus hypocalcemia (5%), toxemia plus hepatic lipidosis (3.4%), and elevated creatine kinase (1.7%). All cases of toxemia had concurrent hyperglycemia. Toxic changes and blood biochemistry changes tended to be higher with a prolonged interval to referral.

This study demonstrates that more severe torsions are more likely to require surgical correction and result in death of the cria. More studies are underway regarding CS in camelids.

Keywords: Uterine torsion, cesarean section, camelid, toxemia

Prevalence and pathologic features of rete testis cysts in alpacas (*Vicugna pacos*)

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Cystic lesions of the rete testis are relatively common in alpacas. The prevalence of these cysts in alpacas is 14.5%, based upon abattoir sampling. This study is a report of prevalence, as well as pathological features of rete testis cysts in alpacas.

Ultrasonography was performed on 173 alpacas scheduled for castration. Cystic dilation of the rete testis was measured. Following castration, rete testis fluid was aspirated and testes and epididymides were fixed and processed routinely for histopathological examination. Sections of testicular parenchyma were evaluated for spermatogenetic activity. Sections of the cauda epididymis were evaluated for presence or absence of spermatozoa.

Rete testis cysts were detected in 32 (18.5%) of the males examined. Cysts ranged from 4 mm to 45 mm (mean ± SEM; 13.3 ± 1.3) in length and from 2 mm to 28 mm (6.5±0.8) in width. The condition was bilateral in 40.6% of the affected males. If unilateral, there was no significant difference ($P > 0.05$) between sides of affected testicles. Small cysts (<10 mm) were located predominately in the center of the testicle, whereas larger cysts occupied the entire length of the mediastinum testis and extended in a branching manner towards the caput epididymis. Fluid aspirated from 44.4% of the cysts contained immature spermatozoa. Immature spermatozoa were obtained significantly more often ($P<0.05$) from larger cysts than from smaller ones.

Spermatogenic activity was in all cystic testicles. However, poor spermatogenic activity was noted in testes with larger cysts. In severe lesions, the most common findings were interstitial edema, enlarged lymphatic vessels and some degenerated tubules. Lumina of the cauda epididymis were completely devoid of spermatozoa in 20% of affected testicles, suggesting complete blockage.

Cystic rete testis may be a significant cause of infertility or subfertility, particularly when cysts are large and extend to the head of the epididymis. The origin of these cysts is not known, but may be congenital, and a hereditary basis is suspected. Ultrasonography of the testicles should be performed as part of any selection or pre-purchase examination of breeding alpaca males.

Keywords: Infertility, reproduction, male

Reference

Sumar J: Studies on reproductive pathology in alpacas [thesis]. Uppsala: Swedish University of Agrarian Sciences;1983.

Ovulation rate in alpacas mated to intact fertile or vasectomized males

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Alpacas are induced ovulators. Ovulation can also be successfully induced with injection of hCG or GnRH. A few alpacas may ovulate without copulation or exogenous hormones. Ovulation has been induced in alpacas by deposition of seminal plasma from alpacas, llamas and bulls, deep into the vagina.[1] The origin of the ovulation inducing factor (OIF) is suspected to be the bulbourethral gland secretions. Our hypothesis is that other factors from the epididymis and testis may be involved in ovulation induction. This study was carried out to compare the ovulation rate obtained following mating to fertile entire or vasectomized males.

Sixty one (61) adult females were selected based on strong receptive behavior 5 days following administration of synthetic PGF2α (fenprostalene, 0.5 mg/head) to 81 alpacas. Females were randomly assigned to two groups; Group 1 (n=31) was mated with intact fertile males and Group 2 (n=30) was mated to laparoscopically vasectomized males. All females were examined by laparoscopy one week post-mating to record follicular and corpus luteum (CL) location, size and number.

The ovulation rate was 87.1 and 76.6% for Group 1 and Group 2 females, respectively ($P>0.05$). The CL sizes (mean ± SEM) were 10 ± 0.5 and 8.5 ± 0.8 mm for a left and right ovarian side in group 1 females, and 9.7 ± 0.7 and 8.1 ± 0.7 mm for the left and right ovarian side in the group 2 females. Double ovulations were recorded in 5 females from Group 1 and 2 females from Group 2. In single ovulators, there were significantly ($P<0.05$) more ovulations from the left ovary (63.9%) than from the right ovary (32.1%). There was no statistically significant difference ($P>0.05$) between the two groups for all parameters studied (ovulation rate, CL size and number).

In conclusion, this trial does not provide evidence that secretions from the testis and epididymis are necessary for ovulation or corpus luteum development. However further studies are warranted because of the sample size.

Keywords: Alpaca, ovulation, reflex, mechanism, OIF

Reference

1. Ríos M, Sumar J, Alarcón V: Presencia de un factor de inducción de la ovulación en el semen de la alpaca, llama y toro [abstract]. Proc. VIII Reunión Científica de la Asociación Peruana de Producción Animal (APPA), 1985. C-276.

Reproductive ultrasonographic imaging in the male Harbor seal

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Introduction

Harbor seals (*Phoco vitulina*) are northern hemisphere phocids with a pupping season from May to July.[1] During part of the captive breeding program at the Oregon Coast Aquarium, the reproductive organs from a male Harbor seal were examined ultrasonographically on three occasions. The research goals were to describe the ultrasonographic appearance of the phocid testes and prostate as well as to compare seasonal changes in size from April to August 2009. The hypotheses were that the ultrasonographic appearance of the reproductive organs in the Harbor seal would be similar to that of the male dog (a domestic carnivore with other reproductive similarities) and that there would be a seasonal increase in reproductive organ size.

Materials and methods

"Q", a ten-year-old captive-born male Harbor seal with no previous fertility information was trained to roll into dorsal recumbency to permit ventral transabdominal ultrasonographic examinations. A Sonoace Pico (Medison Co., Ltd, Seoul, Korea) with a 3.5-5 MHz convex sector probe was used and digital images were captured. Length and width of the testes and prostate were measured using internal calipers. Mean prostatic size was determined by averaging both the length and width measurements; whereas mean testicular size was determined by averaging both the length and width measurements of both testes. One-way ANOVA was performed using GraphPad Prism® (version 4.00 for Windows, GraphPad Software, San Diego, CA) to compare change in testicular and prostatic size between April and August.

Results

The phocid testes had a homogeneous, medium echotexture with a thin hyperechoic peripheral echo. The prostate was moderately echogenic with hypoechoic regions dorsally and ventrally. The prostate capsule was clearly defined dorsally and ventrally but not laterally. Although there was a subtle increase in average prostate size and decrease in average testicular size over time, this change was not significant (Figure 1; $P>0.60$).

Conclusion

This is the first report describing reproductive ultrasonography in a male phocid. The testicular and prostatic echotexture was similar to what has been reported in dogs.[2]

Keywords: Harbor seal, *Phoco vitulina,* prostate, testis, ultrasonography

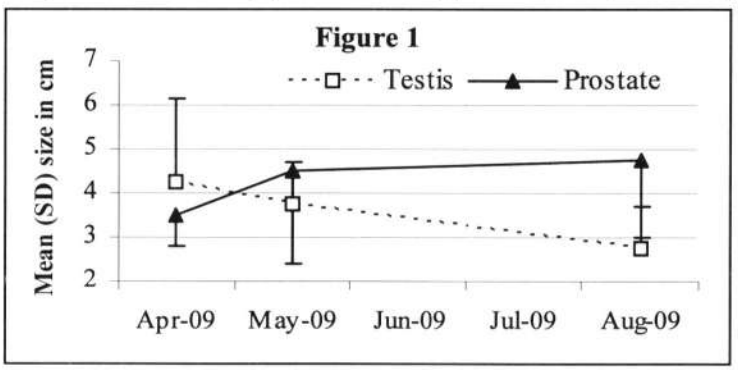

Figure 1. Mean (SD) reproductive organ size during the breeding season in a male Harbor seal.

References

1. Daniel JC: Delayed implantation in the northern fur seal (*Callorhinus ursinus*) and other pinnipeds.J Reprod Fertil Suppl 1981;29:35-50.
2. Davidson AP, Baker TW: Reproductive ultrasound of the dog and tom. Top Companion Anim Med 2009;24:64-70.

Clinical use of recombinant FSH in non-cycling mares

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There are several reproductive states where mares are slow to develop pre-ovulatory follicles or to ovulate (spring transistion, postpartum). Recently a recombinant equine FSH (reFSH) has been used to stimulate follicle development.

The study was conducted on client-owned mares in central Kentucky in 2008 and 2009 and no control treatments were available. Mares were categorized in three groups. Group 1 (n=8) were transitional mares that had not cycled (<20mm follicles). Group 2 (n=9) mares had cycled but later returned to anestrus with follicles <20mm; and Group 3 were post-partum mares that had failed to ovulate.

The criterion for initiation of reFSH treatment was the presence of at least one ≥20 mm follicle. Mares were given twice daily injections of reFSH (0.5 mg per injection IM, AspenBio Pharma, Inc., Castle Rock, CO) until mares acquired one or more ≥35 mm follicles. Twenty-four hours later, 2,500 i.u. of hCG was given to induce ovulation and mares were mated naturally. Mares were examined daily with ultrasound until ovulation. Pregnancy examination was performed 14-16 days after ovulation.

This study tested the hypothesis that reFSH could be used in a clinical setting to induce ovulation in mares failing to develop a pre-ovulatory follicle. The objective was to evaluate the ovarian response of mares in transition and post-partum to reFSH.

Transitional mares were treated in early (Group 1) and late April (Group 2). These mares were treated with reFSH for 6.1 and 4.2 days, respectively. Number of ovulations for Group 1 and 2 mares was 2.6 and 3.1, respectively, and number of pregnancies was 1.9 and 1.7, respectively. Fourteen of 17 transitional mares became pregnant on the cycle after reFSH treatment. Mares in Group 3 (n=6) foaled an average of 44 days prior to reFSH treatment. After 6.5 days of treatment, mares had 3.7 ovulations and 1.8 pregnancies per mare. Five of six mares were pregnant after mating on the reFSH cycle.

In summary, the response to reFSH was similar to that reported previously for eFSH and equine pituitary extract. Recombinant equine FSH was useful to stimulate follicular activity in mares that had "shut down" in late transition and after foaling. Since multiple pregnancies were obtained, management of twins becomes essential after the use of reFSH.

Keywords: Follicle, ovulation, recombinant FSH, pregnancy

Effect of recipient lactation status on pregnancy rate following embryo transfer in alpacas *(Vicugna pacos)*

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Large scale alpaca embryo transfer programs are often hindered by lack of suitable recipients. The objective of the present study is to determine the effect of the lactation status (LS) on the pregnancy rate following the transfer of embryos in alpacas.

A herd of recipients made up of alpacas taken from traditional herds in different communities was placed on natural pastures and given periodic supplementation of oats and alfalfa hay. The body condition score (BCS) ranged from 1.5 to 3.5 (1 to 5 scale). Only females with a BCS ≥ 2.5 were used as recipients. During two breeding seasons (2007 and 2008), 705 embryos were collected non-surgically from an elite herd of 66 females and 19 breeding males. Of these, 54 (7.7%) were discarded after evaluation and 651 were transferred either to alpacas with cria at foot (lactating, n=291) or to non-lactating alpacas (n=360). Recipient follicular activity was monitored by ultrasonography and suitable recipients were induced to ovulate and matched to bred donors when they had a mature follicle (≥ 8 mm and ≤ 12 mm). Ovulation was induced with buserelin (8.4 µg, IM, Conceptal®, Intervet, Lima, Peru). Ovulation was confirmed at the time of transfer by transrectal ultrasonographic visualization of the corpus luteum. All embryos were collected on day 7.4 post-breeding and transferred non-surgically within 20 minutes of collection. Pregnancy diagnosis was performed by ultrasonography eight days post-transfer. Pregnancy rates in lactating and non-lactating alpacas were compared by chi-square analysis. The overall pregnancy rate following transfer was 33%. The pregnancy rates for non-lactating (44.4%) and lactating (18.2%) recipients alpacas were significantly different ($P<0.001$).

These results clearly demonstrate the effect of lactation on pregnancy establishment and maintenance. This effect may be due to negative energy balance and weight loss or other mechanisms that may interfere with corpus luteum function. These factors as well as the effect of nutritional supplementation of lactating recipients under Peruvian pasture conditions on pregnancy rate following embryo transfer are being investigated at present in our laboratory.

Keywords: Embryo transfer, energy balance, pregnancy rate

Genomic variation of uterine isolates of *Streptococcus equi* subspecies *zooepidemicus*

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Introduction

The commensal *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*), the organism most common isolated from uterine infections in mares, displays genetic and antigenic variation which may play a role in pathogenesis and complicate vaccine development. Such variation also challenges researchers to differentiate between experimentally introduced organisms and normal flora. A study was performed to determine the extent of genomic and antigenic variation of uterine isolates of *S. zooepidemicus*, and if isolates collected after experimental infection could be shown to be identical to the inoculated isolate.

Materials and methods

Uterine isolates of *S. zooepidemicus* (n=21) were analyzed by pulsed-field gel electrophoresis (PFGE), and by bactericidal testing in blood of ten horses of varying immunological history. In addition, 13 isolates of *S. zooepidemicus* from fetal and maternal tissues of experimentally infected mares were compared to the inoculated isolate by PFGE. Extraction of genomic DNA followed by restriction endonuclease digestion and PFGE of the DNA fragments was performed as previously described,[1] with the modification that genomes of all isolates were first digested with *Sma* 1, but those with identical *Sma* 1 restriction patterns (pulsotypes) were also digested with *Apa* 1. For bactericidal testing,[1] aliquotted suspensions (250 µL) of each isolate at varying concentrations were prepared, frozen at -80 °C and post-thaw colony counts determined. Suspensions yielding post-thaw counts of 7 to 50 c.f.u per 100 µL were used for bactericidal testing. A 100 µL inoculum of each isolate was incubated in 1.5 mL of fresh blood of a single horse for 48 hours rotated end-over-end at 37 °C. After 48 hours, tubes of blood were either bacteriologically sterile (score = 1) or had been hemolyzed by streptococcal multiplication (score = 0). The procedure was repeated in 10 horses, yielding a specific sequence of 1's and 0's for each isolate across 10 horses. Strain differentiations by bactericidal testing and PFGE were compared.

Results

Isolates yielded 16 different pulsotypes and 13 different bactericidal test sequences. Isolates showing identical pulsotypes showed identical bactericidal test sequences, or sequences different at only one horse. The 13 isolates recovered after experimental infection yielded two pulsotypes, 10 isolates identical to the inoculated isolate, and three isolates of a different pulsotype. Total DNA of both pulsotypes was within 0.5 % (2079.46 kb versus 2088.58 kb), and the variation consistent with a single large inversion mutation of approximately 300 kb.

Discussion

These data indicate that genomic and antigenic variation of *S. zooepidemicus* is widespread among uterine isolates, and is possibly caused, in part, by inversion mutation.

Keywords: *Streptococcus zooepidemicus,* PFGE, bactericidal test, uterus, equine

Reference

1. Causey RC, Weber JA, Emmans EE, et al: The equine immune response to *Streptococcus equi* subspecies *zooepidemicus* during uterine infection. Vet J 2006;172:248-257

Post-dystocia bladder paralysis and cystitis in a mare: medical management and outcome

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A 5-year-old Lusitano mare was referred to the WSU-VTH theriogenology service due to urinary problems 19 days following a dystocia which resulted in delivery of a healthy foal. Transrectal palpation, ultrasonography and vaginoscopy revealed two perivaginal masses, each approximately 7 cm in diameter extending from the vestibular area cranially, as well as a severely distended urinary bladder with an irregular wall contour. Blood work showed elevated BUN, creatinine and normokalemia. A diagnosis of bladder atony due to foaling trauma was made. Empiric therapy was initiated with broad-spectrum antimicrobials and an indwelling urinary catheter. Bethanechol was added as adjunctive treatment for the bladder atony. Specific antimicrobial therapy was started based on urine culture of multi-drug resistant *E. coli* and *Enterococcous* spp. Subsequent bladder lavage and infusion of antimicrobials was performed daily for five days. The mare was discharged 41 days post-dystocia and at six months was clinically healthy.

Foaling has been suspected as a cause of urinary incontinence in horses.[1] However to our knowledge there are no clinical reports documenting this condition and its clinical outcome post-dystocia. Urinary incontinence due to bladder paralysis generally has a poor prognosis[2,3] due to the fact that there is long-standing (months to years) detrusor muscle dysfunction before incontinence becomes a recognizable problem.[4,5] Our case illustrates this delayed clinical recognition, as urinary incontinence took 16 days post-dystocia to develop in this mare. It also demonstrates how important post-dystocia monitoring is and the ramifications of genital lesions on other systems. Equine theriogenologists should remember to monitor the urinary system following dystocia as urinary complications are not rare. Clear communication with clients as to the need of intensive medical management, possible complications and prohibitive cost of care is critical.

Key words: Bladder atony, dystocia, mare, cystitis, bethanechol.

References

1. Schott HC II, Carr EA, Patterson JS, et al: Urinary incontinence in 37 horses. Proc Annu Conv Am Assoc Equine Pract 2004. p. 345-347.
2. Schott HC II: Clinical commentary: urinary incontinence and sabulous urolithiasis: chicken or egg? Equine Vet Educ 2006;18:17-19.
3. Holt PE, Mair TS: Ten cases of bladder paralysis associated with sabulous urolithiasis in horses. Vet Rec 1990;127:108-110.
4. Holt PE, Pearson H: Urolithiasis in the horse-a review of 13 cases. Equine Vet J 1984;16:31-34.
5. Keen JA, Pirie RS: Urinary incontinence associated with sabulous urolithiasis: a series of 4 cases. Equine Vet Educ 2006;18:11-16.

Congenital testicular neoplasia in a two-day-old Holstein calf

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Scrotal enlargement in a calf may be due to inguinal hernia, inflammation, hamartoma, abscess, or neoplasia. Differentiation of the primary problem is paramount to avoid possible intestinal strangulation within an inguinal hernia or loss of valuable genetics from testicular damage from delayed treatment. Physical examination, scrotal palpation, and ultrasound are used to narrow the differential list with histopathology and immunohistochemistry necessary for definitively diagnosis.

In this case a two-day-old Holstein calf presented for a scrotal enlargement. The right testis was firm and bi-lobed with fremitus of the spermatic cord. The physical examination was otherwise unremarkable. Ultrasound examination revealed mixed echogenicity of the stroma and marked dilation of the pampiniform plexus, narrowing the differentials to neoplasia or hamartoma.

Congenital testicular neoplasia in the bovine is rare. Sertoli cell tumors are firm and white with normal to pleomorphic cells.[1] Teratomas, derived from totipotent germ cells, are well differentiated and may contain hair, bone, or teeth. Interstitial cell tumors, the most common in mature bulls, are tan with cells containing abundant cytoplasm, vacuoles, and brown pigment.[1,2] Seminomas consist of polyhedral cells containing a large nucleus with a high mitotic rate, are pinkish, firm, and bulge on cut surface. Hamartomas are non-neoplastic tumors and consist of disorganized hyperplastic mature mesenchymal or epithelial cells within a fibrous stroma.[3]

Bilateral castration was performed and the affected testis consisted of normal appearing, but enlarged testicular tissue ventrally and a fibrous encapsulated mass near the epididymal head. Histopathologically, the encapsulated mass was within the tunica albuginea and subdivided into lobules by collagenous trabeculae obliterating normal cytoarchitecture. Numerous intratubular sheets of polygonal cells with large nuclei, prominent nucleoli, and high mitotic activity were present. The presumptive diagnosis was congenital seminoma. Diagnostic immunohistochemistry is pending.

Keywords: Testis, neoplasia, seminoma, congenital

References

1. Foster R A: Male reproductive system. In: McGavin M, Zchary J, editors. Pathologic basis of veterinary disease. St. Louis: Mosby; 2007. p. 1136.
2. Jensen K, Krag L, Boe-Hansen G, et al: Malignant Sertoli cell tumour in a young Simmenthal bull - clinical and pathological observations. Reprod Domest Anim 2008;43:760-763.
3. Kusewitt D, Rush L: Neoplasia and tumor biology. In: McGavin M, Zchary J, editors. Pathologic basis of veterinary disease. St. Louis: Mosby; 2007. p. 255.

Aspermia and enlarged ampullae following EVA vaccination in a stallion

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A prebreeding season fertility evaluation was performed on a 17-year-old Appaloosa stallion at Oregon State University VTH. The stallion had been bred the previous season, and all mares bred by him became pregnant. The stallion had normal libido when presented to an estrous mare, mounted the phantom and ejaculated on the first attempt. Examination of the ejaculate revealed few sperm with concurrent low semen alkaline phosphatase, consistent with a lack of epididymal secretions. The testes had a turgid consistency with a scrotal width of 10 cm and endocrine results were within normal limits. Transrectal palpation and ultrasonography revealed bilaterally mildly enlarged ampullae. The owner was concerned that the cause of the stallion's aspermia was the result of EVA vaccination (Arvac®, Fort Dodge Animal Health, Ft. Dodge, IA) two months previously. No EVA was detected in the ejaculate using virus isolation and PCR. Semen bacterial culture yielded minimal growth of α-hemolytic *Streptococcus* sp. and *Corynebacterium* sp., consistent with normal flora. Urethroscopy was performed, revealing an inflamed mass on the seminal colliculus but the ampullary and seminal vesicular openings appeared to be normal and unobstructed. Natural and experimental EVA infection can cause ampullitis in stallions.[1,2] However, it is not known if attenuated infection following immunization with a modified-live EVA vaccine could have caused a temporary ampullitis with aspermia as seen in this stallion.

Keywords: Stallion, ampullitis, equine viral arteritis, aspermia

References

1. Holyoak GR, Giles RS, McCollum WE, et al: Pathological changes associated with equine arteritis virus infection in the reproductive tract in prepubertal and peripubertal colts. J Comp Pathol 1993;109:281-93.
2. Blanchard TL, Varner DD, Hurtgen JP, et al: Bilateral seminal vesiculitis in a stallion. J Am Vet Med Assoc 1988;192:525-6.

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