Abstracts

Testicular asymmetry reported after vasectomy in a miniature pinscher dog

Ammasie Allred, Ahmed Mosallam, Michela Ciccarelli, Muhammad-Salman Waqas

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA

A 1-year, intact male miniature pinscher, was presented for lack of penile erection and testicular asymmetry after elective vasectomy performed at another clinic 2 months before presentation. On physical examination, the only abnormality was testicular asymmetry. On reproductive examination, the penis could be exteriorized to the base of the bulbus glandis without resistance. The left testis (6 x 9 mm) was substantially smaller than the right (12 x 23 mm), and the left spermatic cord was thickened. On ultrasonography, the left testis was hypoechoic; the left epididymis was markedly smaller than the right, and hyperechoic lesions were observed in the left spermatic cord. Color Doppler ultrasonography revealed no blood flow through the left pampiniform plexus and testis. The right testis was normal in size, consistency, and ultrasonographic appearance. As the right epididymis was enlarged on palpation and ultrasonography, *Brucella canis* infection was ruled out by serological testing. Bilateral castration and testicular histopathology were recommended. Although vasectomy can lead to atrophy of the seminiferous epithelium and reduced testicular volume, testicular atrophy in this case was unilateral. It is unknown whether the dog had testicular asymmetry prior to the surgery. The pathology of the left testis and spermatic cord may have resulted from congenital hypoplasia or severe ischemic atrophy following vasectomy due to accidental ligation of the pampiniform plexus. Ischemic necrosis would reduce testosterone production that may explain the lack of penile erections. The owner declined measuring serum testosterone concentrations. The etiology of the right epididymal enlargement remains uncertain and could indicate either a congenital anomaly or a potential complication of vasectomy. Canine vasectomy is generally regarded as an acceptable sterilization procedure that preserves the gonads and hormone production, with rare complications including epididymal distention. Further investigation is underway to determine the underlying cause of this dog's reproductive issues.

Keywords: Testis, atrophy, hypoplasia, spermatic cord, ischemia

First documented case of fetal anasarca in a Karst shepherd dog: a detailed ultrasonographic progression from onset to outcome

Ana Andrejašič,[a] Martina Krofič Žel,[a] Tanja Švara,[b] Mitja Gombač,[b] Maja Pipan[c]

[a]Clinic for Small Animals, Faculty of Veterinary Medicine, University of Ljubljana, Ljubljana, Slovenia,
[b]Institut of Pathology, Wild Animals, Fish and Bees, Faculty of Veterinary Medicine, University of Ljubljana, Ljubljana, Slovenia,
[c]Clinic for Reproduction and Large Animals, Faculty of Veterinary Medicine, University of Ljubljana, Ljubljana, Slovenia

Fetal anasarca is a rare condition characterized by substantial fluid accumulation in fetal subcutaneous tissues and body cavities, causing generalized edema. This report presents a case of fetal anasarca in 4.5-year, healthy, primiparous Karst shepherd dog that was artificially inseminated intravaginally with fresh semen. Dog was regularly vaccinated, including against canine herpesvirus, and dewormed. Pregnancy was confirmed on day 20 after ovulation and ultrasonography was performed on days 25 and 30, followed by weekly monitoring. In 1 of the 6 fetuses, fetal anasarca was first observed on day 37 of pregnancy, initially manifesting as fluid accumulation in the subcutaneous tissue of the neck region. One week later, a pleural effusion was detected in the affected fetus. On day 54 of pregnancy, fluid accumulation had further progressed, involving the pleural cavity and to a lesser extent, the pericardium. Two days before delivery, the fetus had no signs of life. Following progesterone concentrations decrease, cesarian surgery was performed. The affected fetus weighed only 220 grams, notably smaller than its littermates (458.6 ± 34 grams). Severe subcutaneous edema and serous fluid accumulation resulted in marked body deformity, with serous fluid filling the abdominal and thoracic cavities. The lungs, liver, kidneys, spleen, and intestines were severely hypoplastic, and the heart chambers were markedly dilated. The morphology and gross lesions were consistent with fetal anasarca. This case is particularly important as it represents the first documented anasarca in the Karst shepherd dog where the progression of fetal anasarca was ultrasonographically monitored throughout pregnancy. The detailed sequential ultrasonographic observations provided a unique opportunity to monitor the dynamic progression of the disease, emphasizing the critical role of regular prenatal ultrasound examinations in detecting fetal pathologies at an early stage.

Keywords: Fetal anasarca, Karst shepherd, ultrasonography, monitoring

Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723

Sperm peritonitis following transcervical insemination in healthy female dogs

Jennifer Attridge, Xavier Schneider, Jodilee Penn, Philip Thomas

Queensland Veterinary Specialists, Stafford, QLD, Australia

Insemination within the peritoneal cavity has been reported in humans, horses and dogs with complications leading to production of sperm antibodies, anaphylaxis, ascites, peritonitis, formation of abdominal adhesions, and systemic inflammatory response syndrome.[1-4] To the authors' knowledge, sperm peritonitis has not been reported secondary to transcervical insemination (TCI) in dogs.

Case 1: A 11-month, maiden intact female mixed breed presented for breeding management and TCI with fresh semen. Ovulation was identified via serial serum progesterone testing and inseminations were performed 24 hours prior to identified ovulation and 24 hours after ovulation (ovulation deemed progesterone concentration > 5-10 ng/ml).[5] Acutely following the second insemination, dog was noted to be lethargic, hyporexic and reactive to abdominal palpation. Dog presented to the clinic pyrexic (39.4°C), with tachycardia. Transabdominal ultrasonography demonstrated free abdominal fluid at the trigone of bladder and between intestinal loops in the caudal abdomen. Abdominocentesis yielded an effusion containing neutrophils, scant rods and detached heads of sperm on cytology. Exploratory laparotomy was performed to decontaminate the abdomen. A 2-mm hyperemic site of perforation through the serosa was identified at the body of the uterus on mesenteric surface. The mesentery was diffusely hyperemic. Effusion fluid sampled from laparotomy was consistent with abdominocentesis fluid taken prior to surgery and cultured *Orchobactrum anthropi* sp. Dog remained in hospital receiving supportive treatments (intravenous fluid therapy, antibiotics and prokinetics); responded well and was discharged after 48 hours. Dog was confirmed pregnant via transabdominal ultrasonography at 4 weeks after insemination with a singleton. An anatomically normal singleton fetus was delivered via timed and planned cesarian surgery on day 63 after ovulation. At surgery no adhesions or scarring was noted within the peritoneal cavity or tubular tract.

Case 2: A 6.5-year, pluriparous intact female French Bulldog presented for TCI with chilled semen. Dog had a history of 3 previous cesarian surgeries. Ovulation was identified via serial serum progesterone testing and insemination was performed 24 and 48 hours after identified ovulation. Acutely following the second insemination dog was presented with a tense abdomen and ptyalism. Transabdominal ultrasonography demonstrated free abdominal fluid at the trigone of the bladder and the broad ligament was noted to be hyperechoic. Exploratory laparotomy was performed for abdominal decontamination. During laparotomy, a small site of perforation was identified at the ventral body of the uterus at the site of a previous cesarian surgery scar. Cytology of the lavage fluid exhibited sperm. Dog recovered well following surgery and was discharged 6 hours after procedure. Transabdominal ultrasonography at 4 weeks after ovulation revealed 4 fetuses. Dog was presented for timed and planned cesarian surgery, resulting in 4 live anatomically normal pups.

These are the first reported cases of sperm peritonitis secondary to TCI in the dog. Furthermore, both dogs became pregnant despite acute exploratory laparotomy after insemination. Sperm peritonitis and sequalae should be considered a complication and risk of a previously considered safe technique.[1]

Keywords: Dog, sperm peritonitis, transcervical insemination

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Spontaneous ovulations in cats used in a nonsurgical spay study

Sandra Ayres, May Einfalt

Department of Comparative Pathobiology, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA

The underlying hypothesis of this project is that fertility and reproductive hormone production in female cats can decrease or eliminated by reducing/destroying the support cells of the ovaries (granulosa and theca cells). We developed a novel nanocomplex consisting of an antibody-guided lipid nanoparticle carrying an intracellular cytotoxin (saporin). This complex can locate the support cells via antimüllerian hormone 2 receptors (AMHR2), enter the cells, and deposit the cytotoxin to induce apoptosis. This study was not anticipated to impact the percentage of spontaneous ovulations (~ 30-60% can occur normally), as cats are induced ovulators. Spontaneous ovulations will produce ovarian corpora lutea (CLs). The aim of this project was to test the ability of this nanocomplex to reduce/eliminate fertility. The following parameters were evaluated: estrous behavior and blood progesterone concentrations during the study, and histology of postspay ovarian tissue. Comparisons of spontaneous ovulations were not originally planned but were analyzed when differences between control and treated groups were observed. Approximately 1- year, purpose-bred female domestic shorthair cats were purchased from a commercial breeder and divided into a control group (n = 6) and treatment group (n = 6). Cats arrived at the end of the normal nonbreeding season (December) and were housed together as a colony with access to ad lib food and water and enrichment. Cats were

Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723

acclimated for 1 week, then exposed to a nonbreeding photoperiod (8 hours light/16 hours dark) in early January. After 5 weeks of low light exposure, the photoperiod was changed to a breeding season photoperiod (14 hours light/10 hours dark). This long-day light regimen continued for the remaining 4 months of the study. The nanocomplex was intramuscularly given midway through the long-day light period. Blood samples were taken from sedated cats (for progesterone (P_4) concentrations and health parameters) during the short light period, then at 3 points during the long light cycle. Cats were observed daily for signs of estrous behavior (spontaneous rolling, spontaneous treading/lordosis, yowling). There were some differences between control and injected cat behaviors, but none were significant. Histologically, the striking difference was CLs in the ovaries. Five out of 6 control cats compared to 1 out of 6 injected cats exhibited CLs ($p = 0.02921$ using Chi-square analysis). Blood P_4 concentrations were also increased in cats with CLs. There were rare occasions of control cats mounting other cats which could explain some spontaneous ovulations, but we would expect to observe this equally in both groups. In conclusion, it appeared that in a colony situation, normal cats will exhibit spontaneous ovulations, in this case 83.33%. In contrast, only 1 of 6 injected cats produced CLs (16.67%). There is the question of whether the nanocomplex injection prevented/interfered with spontaneous ovulations. Questions remain as to whether the nanocomplex would prevent/reduce normal fertile matings.

Keywords: Cats, ovulation, nonsurgical spay

Microbiota-immune interactions in the vaginal and uterine environments from late pregnancy to early postpartum in dairy cows with endometritis

Ali Bazzazan, Rejean Lefebvre

Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada

Transitional period in dairy cows is characterized by substantial microbial and immunological changes within the reproductive tract. This study aimed to investigate the association between the reproductive tract microbiota and the local innate immune response during the transitional period in dairy cows diagnosed with endometritis. We hypothesized that shifts in the vaginal microbiota before calving influence postpartum uterine microbial composition and immune response, leading to an increased risk of endometritis. A retrospective cohort study was conducted on cows categorized as clinical endometritis (n = 11) and healthy (n = 11). Blood, vaginal fornix mucus and uterine mucus samples were collected at 4-time points: 1-week prepartum (−1w) and at 1 (+1w), 3 (+3w), and 5 (+5w) weeks postpartum. Microbiota composition was analyzed via 16S rRNA sequencing, whereas cytokine concentrations of interleukin 1α (IL-1α), interleukin 8 (IL- 8), and α1-acid glycoprotein (AGP) were quantified using ELISA assays. At +3w, cows with endometritis had higher concentrations of IL-1α (1.05 ± 0.01 pg/ml), IL-8 (0.73 ± 0.14 pg/ml) and AGP (2.76 ng/ml) in the vaginal fornix mucus compared to healthy cows ($p < 0.05$). The microbiota analysis revealed a postpartum shift in bacterial composition, with an increased prevalence of potentially pathogenic species like *Trueperella pyogenes* ($p < 0.05$) in cows diagnosed with endometritis. White blood cell counts peaked at +1w postpartum, coinciding with vaginal innate immune changes. The present findings suggested that inflammatory cytokines and acute phase proteins may have a pivotal role in postpartum reproductive tract immunity. Elevated AGP concentrations indicated a regulatory mechanism that balances inflammation and tissue repair. The +3w postpartum period represents a critical window for evaluating immune responses in dairy cows. Understanding the immunological and microbial interactions could enhance diagnostic and therapeutic strategies for postpartum reproductive disorders in dairy cows. These findings suggested that monitoring vaginal microbiota and inflammatory cytokines prepartum could help identify high-risk cows before clinical signs appear, enabling earlier intervention strategies such as targeted probiotic supplementation or immune modulation therapies to improve reproductive outcomes.

Keywords: Microbiota, innate immunity, cytokines, endometritis, dairy cows, postpartum

Equine dystocia managed with assisted and controlled vaginal delivery

Karis Blankenship, Julie White, Heath King, Darcie Sidelinger

Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Starkville, MS, USA

A 14-year, multiparous Quarter Horse mare, was presented for a dystocia. Mare had previously aborted twin foals and was unintentionally bred by a neighboring stallion on an unknown date. On presentation, the foal's forelimbs were observed protruding from the mare's vulva. Foal was in anterior longitudinal presentation, left dorsoileal position, with lateral deviation of the head to the left with torticollis posture. No signs of fetal viability were detected at this time. Abnormal orientation of the fetus in the birth canal is the most common cause of dystocia in the equid.1 Mare was sedated, and an assisted vaginal delivery was attempted for 20 minutes. This was unsuccessful; mare was transported to a surgical suite, placed under general anesthesia, and positioned in the Trendelenburg position for a controlled vaginal delivery. Foal was delivered, vigorously stimulated, and a heartbeat and corneal reflex were detected. It is estimated that the mare was in stage 2 labor for ~ 6 hours; acceptable time for delivery is between 20-30 minutes.1 A retrospective study indicated that every 10-minute increment past the normal range that a foal is in stage 2 labor, the chance of survival significantly decreased.[1] If the controlled vaginal delivery was unsuccessful, a cesarian surgery would have been indicated.[1,2] Fetotomy is indicated if the foal is declared deceased to protect the mare and her reproductive potential.[1,2] Partial fetotomies did not influence a mare's reproductive future.[3] Mare and foal were transferred to a recovery; anesthetic recovery was uneventful. Mare's fetal membranes were manually removed. Common dystocia complications include retained fetal membranes, uterine prolapse, invagination of the uterine horn, uterine rupture, or rupture of the uterine artery.[2] Mare and foal were later discharged from the hospital.

Keywords: Mare, dystocia, controlled vaginal delivery

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Endometrial polyp identified as a potential cause of infertility in a mare

Helen Brown,[a] Karen Von Dollen,[a,b] Karen Wolfsdorf[a]

[a]Hagyard Equine Medical Institute Lexington, KY, USA
[b]Equine Medical Services, Columbia, MO, USA

A 12-year, multiparous barren Thoroughbred mare, was presented to Hagyard Equine Medical Institute's McGee Fertility Unit for evaluation of an intrauterine mass visible on transrectal ultrasonography. The mare had been bred once, with no pregnancy identified on transrectal ultrasonography on day 14 after ovulation; however, an intrauterine mass was visualized, prompting referral. On transrectal palpation, the mare had poor uterine tone and a freely movable uterus in the abdomen. On transrectal ultrasonography the left ovary had a corpus luteum. The endometrial folds had an increased hyperechogenic surface and an ~ 2 x 2 cm solid mass in the middle of the left horn. The uterus was distended with air and hysteroscopy was performed. Both uterine horns were traversed and the oviductal papillae visualized and within normal limits. A scant volume of translucent mucus was present at the base of the right uterine horn. A sample of this fluid was aspirated for culture and cytology. The mass identified on transrectal ultrasonography was visualized near the base of the left horn. The mass was yellow and attached to the endometrium by a thin pedunculated stalk. The mass was bluntly dissected from its stalk using transcervical manual disruption. Histopathology revealed the mass to consist of a polypoid structure that extended to all cut margins consisting of endometrium. The polyp was lined by ciliated, mid height to tall, columnar epithelium. Regions of the epithelium were ulcerated, replaced by thin to moderately sized streams of fibrous connective tissue, and variably covered by necrotic debris and laminated bands of fibrin, sloughed cells, and acidophilic proteinaceous material. The polyp parenchyma consisted of numerous glands with fibrovascular support. The stroma was diffusely infiltrated with moderate to large numbers of lymphocytes and plasma cells and low to moderate numbers of eosinophils. Rare stromal vessels were thrombosed with fibrin. A large peripheral region of the polyp was necrotic. Endometrial cytology revealed several white blood cells, mostly degenerative cells, light mucus and light debris with few gram-positive cocci in pairs intra- and extracellular. Culture of the fluid grew heavy β-*Streptococcus* sp. and light *Mannheimia haemolytica*. The mare was treated for endometritis post hysteroscopy using uterine lavage with sterile saline, infusion of 30% DMSO in saline, and nitrofurantoin. Endometrial polyps are protruding growths from mucous membranes and have been classified as inflammatory or neoplastic lesions.[1-3] They can be pedunculated or sessile, frequently solitary tumors, generally small but may attain a generous size, with their shape mold to the uterine lumen. They can occur in the uterine horn or body and may protrude through the cervix. Smaller polyps are usually asymptomatic, whereas larger masses may degenerate becoming necrotic, ulcerate, and cause clinical bleeding and infection.[4] Endometrial polyps are very rare in mares, with only two cases of uterine polyps documented.[4,5] This polyp may have affected uterine clearance, embryonic mobility, or provided a nidus for infection. After removal of the polyp and treatment for endometritis, the mare became pregnant producing a live foal.

Keywords: Hysteroscopy, endometrial polyps, uterine mass

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Pregnancy toxemia in a dog

Sara Bucher, Katherine Gilmore Smith, Hannah Porter, Sabrina Cousseau, Carolina Ramos, Sara Lyle, Jose Len

Department of Veterinary Clinical Sciences, Louisiana State University, Baton Rouge, LA, USA

A 2-year, pregnant mixed breed Yorkshire terrier-shih tzu, was referred from a local clinic with history of hypoglycemia. On presentation, the body condition score was 2/9, dog exhibited dull mentation, vomiting, lethargy, and weakness. The temperature, pulse and respiration were unremarkable, mucous membranes were pale and tacky, and capillary refill time was over 2 seconds. Bloodwork revealed hypoglycemia (33 mg/dl), hypoalbuminemia (1.1 g/dl) and anemia (packed cell volume: 32%, red blood cell concentration: 4.31/µl). Abdominal ultrasonography detected 4-6 fetuses with heart rates > 200 beats per minute (bpm). Urinalysis revealed ketonuria (1 mmol/l). The presumptive diagnosis was pregnancy toxemia. Pregnancy termination was recommended, but the owner elected to continue with pregnancy and supportive care in hospital. Pregnancy toxemia occurs when the dam does not receive adequate

nutrition, and the definitive parameters are hypoglycemia, ketonemia, and ketonuria. Dog's treatment plan included an intravenous dextrose bolus (2.5% dextrose [3 ml] in saline [1 ml]) followed by 2.5% dextrose in PlasmaLyte (9 ml/hour, total volume: 45 ml/kg/day) with intravenous 10% calcium gluconate (2 ml once), and intravenous ondansetron (0.5 mg/kg every 8 hours). During hospitalization, dog continued vomiting, developed diarrhea and aborted 2 fetuses. Transabdominal ultrasonography revealed 2-4 fetuses (heart rates > 200 bpm). After 2 nights of hospitalization, blood glucose concentrations stabilized (120 g/dl), but hypocalcemia persisted (ionized calcium: 7.5 mg/dl). The owner elected to continue treatment at home due to financial constraints. On discharge, it was recommended to feed small, frequent portions of pup food and to return to the primary veterinarian if health status deteriorated. Three days after discharge, dog returned due to abortion of more fetuses. Transabdominal ultrasonography confirmed abortion of all fetuses. This case highlighted the critical management of pregnancy toxemia in dogs, emphasizing the importance of adequate nutrition during pregnancy, and client education.

Keywords: Pregnancy toxemia, hypocalcemia, diet, ketonuria

Effects of sinigrin in combination with a low iodine diet on equine fetal development and urine iodine concentration

Claire Card,[a] **Maria Jose Terol Monar,**[a] **Laura Hanni,**[a] **Magdalena Lessmeister,**[a] **Rylee Denomy,**[a] **Bernard Laarveld,**[b] **Nadia Cymbaluk**[a]

[a]Department Large Animal Clinical Sciences, Western College of Veterinary Medicine,
[b]Department Animal and Poultry Science, Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada

Sinigrin, a glucosinolate in *Brassica* family plants, breaks down into antithyroid compounds that cross the placenta in some species, but the effects on equine fetal development are unknown. We hypothesized that feeding mares sinigrin in late pregnancy combined with a low iodine diet interferes with fetal thyroid function and skeletal development. Mares with a mean ± SD age of 9.2 ± 4.4 years (range 3-18 years) were individually fed, from mid-pregnancy to term, an isocaloric and isonitrogenous diet at 2% body weight in kilograms (BWkg) dry matter. Mare groups were: National Research Council (NRC) iodine (Control n = 6), no supplemental iodine (No Iodine n = 5), and no iodine plus sinigrin (Mustard n = 2). Forage contained 0.222 ppm iodine. Concentrate was fed at 2 g BWkg: Control oat pellets contained 4.54 ppm iodine, No iodine, contained 0.34 ppm iodine, and Mustard oats with sinigrin contained < 0.003 ppm iodine. At birth, physical examination findings, ultrasonography corrected thyroid volume BWkg, carpal/tarsal skeletal ossification index (SOI) and free catch urinary iodine concentrations using inductively coupled plasma mass spectrometry were measured. Data were analyzed using Shapiro-Wilk, Chi Square and Kruskal Wallis tests at $p < 0.05$. Control and No Iodine foals had physical examination, radiographic, and thyroid findings within normal limits. Mustard foals had the following clinical findings: a 43 kg colt born on day 343 of pregnancy, hypothermia (36.5°C), SOI 1, severe mandibular prognathism, severe forelimb contracture, an enlarged umbilicus, and goiter; and a 32 kg foal born on day 320 days of pregnancy, SOI 3, mild mandibular prognathism, mild forelimb contracture, and thyroid enlargement. Median (quartiles) foal urine iodine concentrations (µg/l) on day 1 were as follows: Control 290 (160, 455), No Iodine 69.6 (23,107.8) and Mustard 7.5 (6.8, 8.3) and were different ($p = 0.04$). The data indicated that sinigrin crossed the equine placenta, interfered with iodine uptake and disrupted fetal thyroid function. The lack of any clinical signs in the No Iodine foals indicated that low maternal iodine intake was insufficient to cause clinical disease, and although numbers are small, the addition of sinigrin produced signs compatible with mild to severe congenital hypothyroidism dysmaturity syndrome.

Keywords: Sinigrin, foal, thyroid, iodine

Uterine transcriptome during parturition in the dog

Gabriela Carneiro de Sousa,[a] **Fernando Biase,**[b] **Bruce Christensen,**[c] **Alyssa Shelby,**[c] **Orsolya Balogh**[a]

[a]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA
[b]School of Animal and Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[c]Kokopelli Veterinary Center, Sacramento, CA, USA

The molecular basis of uterine events leading up to and through the parturition cascade have not yet been fully unraveled in dogs. We hypothesized that global gene expression analysis will pinpoint crucial biological functions (e.g. inflammatory and immune response, steroid hormone- mediated and contractility-associated processes) that will elucidate how uterus prepares for and progresses through labor. Aim of this study was, therefore, to perform RNA sequencing (RNAseq) on canine uterine samples to determine differentially expressed genes and functional pathways from term prepartum through second stage labor. Full-thickness uterine biopsies were collected during cesarian surgery from female dogs divided into 3 groups based on serum progesterone concentrations (P_4) and clinical presentation: planned cesarian surgery (PCS) at term pregnancy without first stage labor signs (n = 7, $P_4 \geq 3.4$ ng/ml); elective cesarian surgery (ECS) at term pregnancy after temperature drop and/or first stage labor signs (n = 6, $P_4 < 1.5$ ng/ml); obstructive dystocia (OD) at second stage of labor presenting with strong spontaneous abdominal contractions (n = 5). RNA isolation was performed as described,[1] followed by DNase treatment and RNA purification (RNA clean & concentrator, Zymo Research). RNA integrity (RIN) was assessed with a 2100 Bioanalyzer Instrument and RNA 6000 Pico Kit (Agilent). Samples included in the study had RIN between 7.4-9.7. Differential transcript abundance for contrasts PCS versus OD, PCS versus ECS, and ECS versus OD were determined by employing the quasi-likelihood negative binomial generalized log-linear model from the R package 'edgeR'[2] and the Wald test from the R package 'DESeq2'.[3] False Discovery Rate (FDR)[4] threshold was set < 0.05 in both tests for a gene to be differentially expressed (DEG). There were 4 and 5 genes with transcripts exclusively detected in OD and ECS samples, respectively. A total of 541 DEGs were identified for PCS versus ECS, 3443 DEGs for PCS versus OD, and 10 DEGs for ECS versus OD. After filtering

gene ontology terms by FDR < 0.1 and at least 6 genes per category, preliminary analysis for PCS versus ECS contrast highlighted changes in biological and cellular processes such as angiogenesis, positive regulation of cell migration and cell population proliferation, positive regulation of phosphatidylinositol 3-kinase/protein kinase B signal transduction, cellular response to hypoxia, and negative regulation of apoptotic process. In the PCS versus OD contrast, there were DEGs involved in functions that were overlapping with the PCS versus ECS contrast, and additionally, other processes such as actin cytoskeleton organization, G protein-coupled receptor signaling, inflammatory response and immune system process, and carbohydrate transport that appeared to be more characteristic of the actively contracting uterus during second stage labor. These preliminary results provided insights into a broad range of biological and molecular processes that regulate uterine function during the parturition process in the dog.

Keywords: Pregnancy, parturition, uterus, gene expression, dog

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Rectovaginal fistula in an adult English bulldog

Gabriela Carneiro de Sousa, Julie Cecere, Orsolya Balogh

Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

A 2-year, spayed female English bulldog was referred to our clinic for vaginitis and recurrent presence of fecal material within the vestibule after urination. After ovariohysterectomy surgery, weekly episodes of painful fecal eliminations with straining to urinate were noted. Accumulation of gritty material in and around the vestibule and vulva immediately after urination were observed. Physical examination was unremarkable except for slight erythema around a moderately recessed vulva. Digital rectal palpation revealed firm fecal material and a 2 cm diameter sparsely covered diverticulum on the ventral rectal wall about 3 cm cranial from a normal anus. Vaginal palpation revealed a 360° fibrous vestibulovaginal stricture and a midline dorsoventral band. No communication between the rectum and the vagina was evident when rectal and vaginal palpation was performed simultaneously. Urinary tract infection was ruled out by urinalysis and culture. Vaginitis was diagnosed via cytology containing scant bacteria and a moderate number of neutrophils. Vaginoscopy using a rigid endoscope and air insufflation confirmed a midline dorsoventral band just cranial to the urethral papilla, severely erythematous mucosa of the vestibule and vagina and numerous multifocal lymphoid follicles. There were 2 fibrous tissue bands on both lateral vaginal walls. The fibrous tissue band on the left surrounded a small diverticulum with thin mucosa, and within the diverticulum there was a small round-to-oval area of very thin mucosa or fibrous tissue. No grossly visible communication between the fistula and the rectum was seen; however, air was observed escaping out of the rectum. Based on history, physical examination, and vaginoscopy findings, we diagnosed this dog with rectovaginal fistula, although its etiology, i.e. congenital or acquired, could not be reliably determined. The owner opted for nonsurgical treatment with oral lactulose (1 ml) every 12 hours and nonsteroidal anti-inflammatory therapy with oral carprofen (2.2 mg/kg) every 12 hours for 3-5 days. At the time of this report, the dog is still on lactulose, she is comfortable defecating, and the episodes of fecal material within the vestibule reduced greatly, but still occur every couple of weeks. Rectovaginal or rectovestibular fistula is an uncommon congenital or acquired defect in the ventral wall of the rectum and the dorsal wall of the vagina or vestibule that allow the passage of feces through the fistula. The condition has been reported as a single abnormality[1] or in conjunction with atresia ani and other developmental abnormalities.[2,3] Clinical signs usually become evident within the first few weeks after weaning when diet changes lead to firmer fecal consistency.[4] Surgical correction is curative and is the treatment of choice.[1,4] However, complications such as wound dehiscence, surgical reintervention, perineal swelling, fecal incontinence and recurrent constipation have been reported.[1,5] Surgical correction will be considered as the next step for this patient if her clinical signs worsen. In conclusion, nonsurgical management of the rectovaginal fistula decreased the episodes of fecal passage into the vestibule and improved the quality of life of the dog.

Keywords: Rectovestibular fistula, rectovaginal fistula, dog, vaginitis, vaginoscopy

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Cooling rate effect on postwarming outcomes from bovine embryo vitrification

Soon Hon Cheong,[a] **Abdallah Abdelhady,**[a] **Jingzhi Zhang,**[b] **ChiaKuan Wu,**[b] **Yoke Lee Lee,**[a] **Meihong Shi,**[b] **Patrick Crane,**[a] **Ian Porter,**[a] **Mariana Diel de Amorim,**[a] **Robert Thorne,**[c] **Jingyue Duan**[b]

[a]Department of Clinical Sciences, College of Veterinary Medicine, [b]Department of Animal Science, College of Agriculture and Life Science,
[c]Department of Physics, College of Arts and Sciences, Cornell University, Ithaca, NY, USA

Vitrification is a crucial technology to expand the use of embryo transfer but it reduces embryo viability. Cryodamage from vitrification is primarily caused by intracellular ice forming at cooling and growth during warming, which is influenced by cooling rate. We hypothesized that increasing cooling rate from the industry standard cooling (SC) rate (~ 23,000°C/minute) to ultra-fast cooling (UFC) rate (~ 600,000°C/minute) eliminates intracellular ice and improves postwarming outcomes. Aim of the study was to compare intracellular ice from cooling and ice growth during warming, using time-resolved synchrotron x-ray diffraction and to compare postwarming outcomes including reexpansion, hatching, apoptosis, and gene expression changes. Abattoir- derived bovine embryos were randomly assigned to 1 of 3 treatment groups: SC, UFC, and unvitrified control (CTL). Intracellular ice was detected and quantified using a custom synchrotron x-ray diffraction data analysis pipeline. Both SC and UFC vitrified embryos without detectable intracellular ice from cooling, but only SC had ice growth at warming detected using time-resolved synchrotron x-ray diffraction. Postwarming outcome was analyzed using time-lapse videography and differences among groups evaluated using ANOVA or Cox's Proportional Hazard models in JMP Pro v.18. CTL embryos (n = 100) had the fastest median re-expansion time 181 minutes (95% CI 142-211 minutes) compared to UFC embryos (n = 71) (226 minutes, 95% CI 191-260 minutes), which were faster than SC embryos (n = 76) (491 minutes, 95% CI 441-561 minutes). Embryo hatching rate was similar between control (92.0%) and UFC (87.3%) but higher than SC (77.6%) and the median time to hatching was faster for CTL (1,215 minutes, 95% CI 1,073-1,353 minutes) and UFC (1,153 minutes, 95% CI 1,014-1,394 minutes) compared to SC embryos (1,808 minutes, 95% CI 1,615-2,055 minutes). Apoptosis was evaluated using TUNEL staining and the proportion of apoptotic cells was higher for SC (7.3 ± 0.6%) compared to UFC (3.5 ± 0.3%) and CTL (4.1 ± 0.7%). Transcriptomic analysis was performed on 3 replicates of pooled embryos and cluster analysis showed CTL having some overlap with UFC, whereas SC had a more distinct profile compared to CTL. We identified 110 differentially expressed genes (DEGs) in SC versus CTL with downregulation of genes associated with adhesion and cell-junction assembly, whereas DNA- damage repair pathway was upregulated. UFC and CTL comparisons identified 72 DEGs with upregulation in lipid metabolism and carboxylic acid biosynthesis and downregulation of secondary-messenger mediated signaling and cell migration. SC versus UFC comparison identified 100 DEGs with upregulation of signal transduction in response to DNA damage, lipid transport and localization and upregulation of system process, neuron differentiation and guidance. Increasing cooling rate to UFC eliminated ice formation and improved postwarming embryo outcomes as well as minimized gene expression changes related to cryodamage.

Keywords: Vitrification, cattle, embryo

Rheotaxis-based sperm separation of frozen-thawed equine semen using microfluidics

Soon Hon Cheong,[a] **Mohammad Yaghoobi,**[b] **Morgan Rollins,**[a] **Alireza Abbaspourrad**[b]

[a]Department of Clinical Sciences, College of Veterinary Medicine, [b]Department of Food Science, College of Agriculture and Life Science, Cornell University, Ithaca, NY, USA

Rheotaxis is a natural behavior of sperm orienting and swimming against fluid flow. Female reproductive tract has fluid flowing from the uterine tubes to the vagina and rheotaxis may be a natural method for selecting superior sperm for fertilization. Objective was to determine if sperm selection by rheotaxis can improve the quality of frozen-thawed stallion semen. Purpose of this study is to test the hypothesis that rheotaxis-based sperm separation improves the quality of frozen- thawed stallion semen samples compared to unselected raw samples or selection by density gradient centrifugation. Frozen-thawed semen from 3 stallions of varying fertility was used for this study and the pooled sample was divided into 5 groups: control (CTL) group was not processed and left in a tube until analysis, density gradient centrifugation (DGC) group was processed with a single layer EquiPure (Nidacon) at 300 g for 30 minutes, and rheotaxis-based separation using a microfluidic chip was performed at 3 flow rates, 150 µl/hour (150 µl), 250 µl/hour (250 µl), and 350 µl/hour (350 µl). Recovered samples were evaluated for total concentration and membrane damaged sperm using a Nucleocounter (Chemomatec) and motility parameters were evaluated using a Computer Assisted Sperm Analysis system (CEROS, Hamilton Thorne). Differences between groups were determined using ANOVA and Student's t post-hoc test with the CTL group used as the reference. Progressive motility was different (p = 0.0102) among groups with the CTL group (32.5 ± 4.2%) having the lowest motility that was not different from the DGC (41.7 ± 16.0%) group, whereas the rheotaxis groups were higher than the CTL with 59.3 ± 12.4%, 56.25 ± 5.1%, 55.0 ± 3.1% for the 150 µl, 250 µl, and 350 µl groups respectively. Motility in the 150 µl group was also higher compared with the DGC group. The proportion of membrane damaged sperm was significantly different between groups (p = 0.0124) and was greater in the DGC group (45.8 ± 4.3%) compared with the CTL (35.6 ± 5.9%) and rheotaxis groups 150 µl (28.4 ± 4.0%), 250 µl (26.9 ± 1.4%), and 350 µl (32.1 ± 6.4%). In this preliminary study with a small sample size, rheotaxis sperm separation appeared to improve the proportion of progressively motile sperm and potentially superior to EquiPure for this purpose. The proportion of membrane damaged sperm was higher in the EquiPure group compared with all the other groups likely because of centrifugation especially at higher forces. Rheotaxis-based sperm separation has improved bovine in vitro fertilization rates and may be able to improve fertility of equine sperm.

Keywords: Rheotaxis, sperm separation, microfluidics

Disagreement between claimed and actual quality of shipped frozen canine semen

Bruce Christensen,[a] **Hollis Erb,**[b] **Kinsey Smith,**[a] **Alyssa Shelby**[a]

[a]Kokopelli Veterinary Center, Sacramento, CA, USA
[b]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University Ithaca, NY, USA

Cryopreserved semen is commonly stored for long periods and shipped to facilities all over the world to preserve genetics over time and space in canine breeding programs. We compared the postthaw motility and overall sperm number in frozen/thawed ejaculates (n = 147) with what was claimed on the records accompanying the shipped ejaculate. Samples from international sources or any cryopreserved in our own laboratory were excluded. Cryopreserved semen was received at our facility from other facilities (n = 43) all over the US and promptly, quickly transferred to a liquid nitrogen storage tank where it was maintained until needed for insemination. Cryopreserved semen was thawed at 37°C in a water bath (straws) or thaw media (pellets). Any defective straws were excluded from analysis. A subsample of the thawed ejaculate was incubated in thaw media (if an adequate volume was provided by the shipping institution) or otherwise in CaniPlus AI solution (Minitube USA), for 5 minutes prior to evaluation. Sperm concentration and total motility were objectively measured using standardized protocols on a SpermVision computer system (Minitube USA), supervised by 1 of 2 investigators (BWC, n = 74; AS, n = 73). Claimed and recorded values were compared using Wilcoxon's signed-rank test. Claimed total number of sperm was included in records from 97/147 cases, of which 75% overreported by at least 16 x 10^6 sperm and 50% overreported by at least 97 x 10^6 sperm. Claimed postthaw motility was included in records from 135/147 cases, of which 3/4 overreported by at least 24% and half overreported by at least 37%. The differences between claimed and objective results were significant for both total sperm number and total motility ($p < 0.0001$, in both cases). We used a sliding scale (ranging from 50-200 x 10^6 motile sperm) based on 5 weight classes to determine a minimum, adequate breeding dose. In only 2/147 cases did the shipped dose met the minimum criteria. In some cases, multiple recommended insemination 'doses' were shipped, allowing us to thaw more than the initially recommended amount in an attempt to reach a true recommended minimum dose that we achieved in 44 additional cases. For those cases in which pregnancy data exist (n = 132), 80% of dogs that received an adequate insemination dose (n = 46) were diagnosed pregnant compared to a 69% pregnancy rate in those dogs that received a less than adequate insemination dose (n = 86). These comparisons highlighted the dire state of the canine frozen semen industry in the US. Responsible dog breeders invest much in the way of time, money, and emotion into decisions to preserve their dogs' genetics for future use. Measures taken to protect the consumer by demanding better training, transparency, and oversight in the practice of canine semen cryopreservation would serve clients better.

Keywords: Semen, cryopreservation, canine, breeding, oversight

Analytical validation of different diagnostic tests for the detection of leukocytes in canine semen

Daniela Cortes,[a] **Maria Ferrer,**[a] **Roberto Palomares,**[a] **Claudia Escobar,**[b] **Robyn Ellerbrock**[a]

[a]Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA, USA
[b]Department of Animal Health, College of Veterinary Medicine, National University of Colombia, Bogota, Colombia

Leukospermia in dogs is defined as an abnormal concentration of white blood cells (WBC) in the ejaculate (> 2,000 WBC/µl).[1] Occasionally, disorders associated with the prostate such as prostatitis are responsible for this finding.[2,3] The most frequently used tool to identify WBC in semen is cytology. However, the morphological differentiation between germ cells and WCB is often challenging. Objectives of this study were to validate 5 diagnostic tests for leukospermia (hemacytometer, leukocyte esterase dipstick test, peroxidase stain, semen cytology, and immunostaining CD45+) and to evaluate the effect of varying concentrations of WBC on sperm motility parameters. We hypothesized that all diagnostic tests have a diagnostic value and that > 2.5 x 10^6 WBCs/ml affects motility parameters. A total of 9 semen samples from 7 healthy sexually mature adult dogs were analyzed. Leukocytes were purified from autologous blood samples by density centrifugation. Leukospermia was induced in aliquots of 50 x 10^6 purified sperm in different concentrations: negative control (NEG = no WBC, only sperm), positive control (POS = no sperm, only WBC), treatment 1% (T1% = 0.5 × 10^6 WBCs/ml), treatment 5% (T5% = 2.5 × 10^6 WBCs/ml), and treatment 15% (T15% = 7.5 × 10^6 WBCs/ml). Total and progressive motility were evaluated 0, 24, and 48 hours after induction of leukospermia at 37°C. Five diagnostic tests were used to quantify concentrations of WBC in each sample: hemacytometer, leukocyte esterase dipstick test, peroxidase test, semen cytology, and immunostaining CD45+. The median number of cells counted in the hemacytometer, peroxidase test, and cytology was significantly higher in T5 and T15% than in NEG, whereas the CD45 immunolabeling was higher in T15% than in the NEG group (Kruskal-Wallis test with a Dunn's post-hoc test; $p < 0.05$). Concentrations of WBC detected with the leukocyte esterase dipstick test was different among treatments (Chi square test; $p < 0.05$). Both 5 and 15% of WBC in the ejaculate lowered sperm total and progressive motility at time 0. By 24 hours, total and progressive motility were only different between control and T15%, but by 48 hours, all treatment groups had lower total and progressive motility than the control group ($p < 0.05$). Thus, samples in T5 and T15% were considered positive for leukospermia. Peroxidase test had the best analytical sensitivity (96.3%) followed by cytology (92.6%). Peroxidase test and leukocyte esterase dipstick had the lowest analytical specificity (88.9%). Cytology had the highest positive predictive value and positive likelihood ratio (96.2% and 16.67, respectively) and the peroxidase test had the highest negative predictive value and the lower negative likelihood ratio (94.1% and 0.04, respectively). As hypothesized samples with ≥ 2.5 x 10^6 WBCs/ml had impaired motility parameters at times 0, 24, and 48 hours after induction of leukospermia. Both cytology and peroxidase test were considered the ideal methods to diagnose induced leukospermia in purified semen samples in the dog.

Keywords: Dog, semen, round cells, leucocytes, leukospermia

Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723

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Transcriptomic profile of single immature and in vitro matured equine oocytes after holding

Sabrina Cousseau,[a] Victor Medina,[a] Carlos Neto,[b] Michala Lindley,[b] Shavahn Loux,[c] Matheus Azevedo,[b] Daniel Rosseti,[b] Mark Mitchell,[a] Carlos Pinto[d]

[a]Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
[b]Genetech Animal Reproduction, Purcell, OK, USA
[c]Department of Equine Physiology and Bioinformatics, School of Animal Sciences, Louisiana State University, Baton Rouge, LA, USA
[d]Department of Ambulatory Medicine and Theriogenology, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA

Previous studies have observed a progression in chromatin condensation during holding of equine cumulus-oocyte-complexes (COCs) at room temperature. However, the impact of holding on the transcriptome of these gametes has yet to be determined. This study aimed to characterize the effects of holding COCs at a controlled room temperature (22°C) on the transcriptomic profile of immature and in vitro matured (IVM) oocytes. We hypothesized that holding COCs is associated with progression into transcriptional silencing. A total of 125 compact COCs were collected from 37 mares through transvaginal aspirations of follicles ≤ 25 mm in diameter. Only COCs with a minimum of 3 layers of cumulus cells were selected and allotted into 4 groups: 1- CT (n = 20), processed at collection; 2- H (n = 30), held in commercial embryo holding media (ABT Holding, ABT 360) at 22°C for 24 hours; 3- IMM-IVM (n = 40), placed immediately into IVM for 30 hours; and 4- H-IVM (n = 35), placed into IVM for 30 hours after holding for 24 hours. COCs were denuded at various time points and stored in liquid nitrogen. Single oocyte RNA extractions, concentration, and quality assessments were performed, leading to 33 samples (CT: n = 9; H: n = 8; IMM-IVM: n = 8 metaphase II; H-IVM: n = 8 metaphase II) being selected for library preparation and sequencing (150 pb-PE). Reads were trimmed, mapped (equine reference genome, ECab 3.0), and quantified using featureCount, with similar read counts observed among oocytes. A total of 13,263 genes were analyzed. As expected, only 2 differentially expressed genes (DEGs) were observed between CT and H oocytes, being upregulated, whereas the number of transcripts was not different among groups for the reminder of genes. Moreover, there were only 24 DEGs in mature oocytes (IMM-IVM and H-IVM) and all were upregulated. Gene ontology analysis indicated an increase in protein ubiquitination during holding that has also been described as a component of chromatin condensation pathways. For mature oocytes, DEGs were associated with processes such as chromatin and chromosome organization, microtubule polymerization, regulation of transcription, translation, and protein assembly. These results suggested that a possible progression into transcriptional silencing occurs once equine COCs are removed from the follicular environment and placed in holding media, with minimal transcriptional activity accounting for chromatin changes observed in studies.

Keywords: Oocyte, holding, transcriptome, horse

Successful uterine prolapse replacement of extended duration in a Hereford cross cow

Cody Davis, Jessica Klabnik, Jessica Rush

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

A 2-year, Hereford cross cow, was presented for evaluation of a postpartum uterine prolapse over 48 hours in duration. Parturition was otherwise uneventful, and the calf was apparently healthy at presentation. The owner replaced the uterine horn in the vagina but failed to reinvert the horn. Subsequently, the cow ruptured through the retention stitches the owner placed. Examination revealed a torn and necrotic vulva consistent with trauma from prolapsing through sutures, and the exposed uterine horn was relatively nonpliable with areas of necrosis. A caudal epidural with 2% lidocaine (100 mg) was given and the exteriorized tissue was cleaned with soap and water. Brown roll gauze was used to manually reduce uterine edema and size with slight effect such that the uterus was unable to be replaced. Intramuscular oxytocin (100 IU) was given and was successful in reducing the size of the uterus, so additional intramuscular oxytocin (200 IU) was given with further size reduction achieved, allowing the horn to be reinverted. This action is contrary to current guidance recommending avoiding the use of oxytocin until after the uterus is successfully replaced.[1] The uterine horn was then successfully inverted and replaced using a wiffle ball bat. During inversion and replacement, large quantities of urine were expelled. Umbilical tape sutures were placed in a horizontal mattress pattern, and transdermal flunixin meglumine was given. Overnight observation demonstrated the appropriate ability to urinate. No apparent complications were reported. Given the extended duration of uterine prolapse and time of the uterine horn exposed to the environment despite owner efforts, it is notable that this case was able resolved with no immediate mortality due to necrosis or fatal hemorrhage due to uterine vessel rupture.[1]

Keywords: Prolapse, uterine, oxytocin, necrosis, postpartum

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Uterine environment reduces the pluripotency of the in vitro-produced blastocyst

Alejandro de la Fuente, Soledad Martin-Pelaez, Kornelia Omyla, Kazuki Takahashi, Jamie Norris, Pouya Dini

Department of Population, Health and Reproduction, University of California, Davis, Davis, CA, USA

With the increasing production of equine embryos in vitro, understanding how in vitro culture affects early cell lineage segregation, compared to uterine signaling in vivo, has become critical for optimizing outcomes in the laboratory and subsequent pregnancy. During early embryo development, the first lineage differentiation occurs as 1 population of cells differentiates into the trophectoderm (TE) and another group of cells into the inner cell mass (ICM), losing their totipotency and becoming pluripotent cells. The timing of these differentiation events, as well as the dispersal and intermingling of cells, may be linked to a higher incidence of monozygotic twinning observed from in vitro-derived embryos when compared to their in vivo counterparts. In this study, we sought to identify specific gene markers differing between 2 sets of embryos produced in vitro (IVP). The first group corresponds to blastocyst stage embryos vitrified, thawed, and then in vitro cultured for an additional 48 hours (TC). The second group corresponds to blastocyst stage embryos vitrified, thawed, transferred into recipients, and flushed 48 hours later (ET). Both groups of embryos underwent microscopic evaluation, followed by RNA extraction. The extracted total RNA was sequenced, and the resulting reads were trimmed (Trim Galore) and aligned (STAR) to the current equine reference genome. Mapped reads were then quantified (featureCounts) and analyzed with DESeq2 (FDR cutoff: 0.1; minimum $|\log_2$ fold change$| \geq 2.0$) to identify differentially expressed genes (DEGs). Blastocysts flushed 48 hours after transfer had a distinct ICM, a confluent capsule, and a marked increase in size. TC embryos did not have any distinct changes during the 48 hours of culture. Transcriptomic results revealed large number of DEGs (n = 1,561) between the 2 groups. Notably, there was a downregulation of pluripotency– associated genes (PODXL, PODXL2, NANOG, SOX2, DNMT3B) in transferred embryos. In addition, genes involved in embryo–maternal communication—such as ESR1, CYP19A1, and PTGES2—were upregulated in the transferred group. These findings indicated that the uterine environment provides a distinct set of signals that promote differentiation of in vitro-produced embryos and diminish pluripotency in the developing embryo as the ICM initiates the lineage of cells that will transform into the embryo paper.

Clinical workup of a hyperechoic structure in the uterus of a chronically infertile mare

Dylan Desosa

Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Hyperechoic images in the uterus of the mare can include air, foreign objects (culture swab tips, marbles), tissue debris, medications (ampicillin, gentamicin), urine, blood, mucus, retained fetal membranes, endometrial cups, and fetal bones.[1] A 10-year, Quarter Horse mare, was presented in April, 2024 for a reproductive examination due to chronic infertility. The mare aborted a 5-month fetus in October 2022 and had not been rebred in 2023. Before admission in 2024, the mare was bred during 2 estrous cycles, and the referring veterinarian noticed by transrectal ultrasonography a hyperechoic image at the base of the right uterine horn that persisted following multiple uterine lavages. The lavage fluid was positive for *Escherichia coli*. Transrectal palpation/ultrasonography evaluation of the reproductive tract indicated moderate uterine edema, ~ 8 cm of intrauterine fluid, and an elongated hyperechoic structure in the lumen of the left uterine horn that was palpably hard. Purulent fluid, containing numerous cocci and neutrophils, was lavaged from the uterus. The following day, uterine endoscopy was performed, and an irregularly shaped, white-to-tan hard structure (resembling a 4 cm pelvic bone) in the lumen of the left uterine horn was identified and removed. Retained fetal bones can occur because of fetal death and result in maceration (which is associated with bacterial contamination [likely this case])[2] or mummification.[3] This mare aborted what appeared to be an intact single fetus 18 months before admission, so this would have been the only opportunity for the fetal bone to be retained, possibly due to an unrecognized retained twin. This case highlighted the diagnostic challenge of ultrasonographic 'hyperechoic' images and the eventual diagnosis, in this case, of fetal bone. In addition, following abortion, the uterus should be examined for the presence of a twin fetus.

Keywords: Horse, uterus, fetal maceration, retained bone

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Chronic balanoposthitis and urethritis secondary to phimosis in a Tennessee Walking Horse gelding

Jack Detten, Dustin Major, Mariah Pearson, Sophia Marchio, Yatta Boakari

Department of Large Animal Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX, USA

A 22-year, Tennessee Walking Horse gelding, was presented for a 16 month history of phimosis[1] and urination within the sheath. Gelding also had a 2 month history of urethritis and balanoposthitis. Initial management prior to referral included 2 smegma transplantations and urethral endoscopy that identified a hyperemic distal urethra. Gelding failed to

improve and was referred for a phallectomy. Clinical evaluation revealed a white caseous, crusted discharge on the prepuce, surrounding skin, and inner hindlimbs, ulcerations on the distal penis, thick and hyperplastic glans penis, and a hyperemic, inflamed distal urethra. Preoperative bloodwork revealed a mild leukopenia (4.9 x 10^3 cells/µl) but no other clinically substantial abnormalities. A sheath ablation and en bloc resection with total phallectomy was performed using the Williams technique.[2] Surgery was performed under general anesthesia following a caudal epidural and pudendal nerve block, and preoperative and postoperative antibiotics were given. The dorsal aspect of the penis was tacked to the body wall and a Jackson-Pratt drain was placed. A 25 x 17 x 5 cm segment of prepuce and distal penis was submitted for histopathology. Histology findings were consistent with chronic, moderate, multifocal to coalescing, ulcerative, lymphoplasmacytic balanoposthitis with granulomatous folliculitis with furunculosis and dermal fibrosis. These results confirmed the clinical diagnosis of chronic balanoposthitis. No evidence of neoplasia or dysplasia was noted. The patient remained hospitalized for 5 days after surgery and was discharged as planned. Phimosis is classified as either congenital or acquired. Acquired phimosis is a common sequela of local neoplasm or trauma.[1] Surgical management is often the only treatment option.[3] In this case, the chronic balanoposthitis likely resulted from dermal injury by recurring urination within the sheath. Due to the chronicity of the inflammation and consequential urethral stenosis, surgical management was indicated with a positive result that increased the gelding's quality of life.

Keywords: Phimosis, chronic balanoposthitis, urethritis, gelding, ultrasonography, phallectomy

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Paraphimosis in a Thoroughbred gelding

Katherine Dickinson, Hannah Maxwell, Dane Schwartz, Aime Johnson

Department of Clinical Sciences, Auburn College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Paraphimosis is the inability to retract the penis into its normal position in the prepuce. As the penis protrudes, the muscle fatigue, swelling, and stretching of the pudendal nerves leads to the inability of the horse to retract the penis. The exposed epithelium becomes inflamed and friable, predisposing the underlying loose connective tissue to bacterial infection. This infection leads to fibrosis and paralysis that permanently inhibit the penis to retract back into the prepuce.[1] However, paraphimosis can be resolved, and normal function can be restored if treated appropriately and promptly. An 8-year, Thoroughbred gelding, was presented for paraphimosis. The paraphimosis could have been caused by trauma, but a perceived weight loss in the gelding was observed each winter. Body condition score was 2/9; gelding's penis had severe edema with moderate excoriations. Hydrotherapy was performed, followed by an application of Femycin and silver sulfadiazine cream on the extended penis and prepuce. A few hours later, the penis was wrapped with brown gauze and an Esmarch bandage for 15 minutes. A purse string suture was placed around the preputial opening. Once the compression bandage was removed, the penis and prepuce were manually replaced into the preputial cavity. The purse string was tightened to contain the penis, leaving 1 finger width allowing for urination. The following day, the purse string was removed and replaced by a customized bottle to secure the penis in the preputial cavity, tying rubber bands around the gelding's back to keep it in place. During the gelding's 5-day hospitalization, hydrotherapy was conducted twice daily with daily penile compression to reduce edema. Anti-inflammatories and antibiotics were sent home with the bottle to keep on until the gelding could maintain the penis within the prepuce without assistance.

Keywords: Horse, paraphimosis, penis, penile paralysis, gelding

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Cataloguing G-protein coupled receptors expressed in the canine placenta

Jamie Douglas,[a] Viviane Gomes,[b] Chelsey Yob,[c] Dalen Agnew[c]

[a]Comparative Medicine and Integrative Biology, [b]Department of Small Animal Clinical Sciences, [c]Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA

G-protein coupled receptors (GPCRs) are the largest family of mammalian cell surface receptors, more than half functioning in olfaction. The remaining nonolfactory GPCRs (endoGPCRs) modulate physiologic processes and serve as key pharmaceutical targets. Little is known about expression and function of GPCRs in the canine placenta. The dog genome contains genes for ~ 1438 GPCRs, 353 of which are classified as the endoGPCR subtype. Our project aimed to characterize the endoGPCRs present in the canine preterm and term placenta. We hypothesized that GPCR spatial gene expression is influenced by sex, placental anatomy, breed, and maternal fitness. Placental samples were collected from 12 canine litters following elective cesarian surgery. Placentas from at least 1 male and 1 female were processed per litter. Tissue was sampled from each of the 3 regions of the zonary placenta (allantochorion, transfer zone, hematogenous zone) and preserved in RNAlater (Thermo Fisher Scientific) at –80°C until RNA extraction. Demographics of sampled litters

were term golden retriever (n = 3), term French bulldog (n = 3), 1 week premature fit Beagle (n = 3), and 1 week premature obese Beagle (n = 3). Extracted RNA samples (n = 72) underwent mRNA-sequencing and subsequent bioinformatic analyses, resultant data providing the repertoire of the endoGPCRs expressed in the canine placenta as influenced by sex (male versus female), placental anatomy (allantochorion versus transfer zone versus hematogenous zone), temporal change (term versus preterm), and metabolic state of the dam (fit versus obese). Data generated by this research serve as foundation for future investigation of canine placental endoGPCRs in the context of pregnancy disorders, such as dystocia, maternal health, and birth defect. Ideally, design and outcomes of future GPCR exploration will aid in development of veterinary therapies to improve pregnancy outcomes for the bitch and neonate.

Keywords: G-protein, receptor, mRNA-seq, dog, placenta

Decoding hormonal effects in bovine oviductal organoids: a new era in reproductive research

Brandi Dunn,[a] Ahmed Gad,[b] Riley Thompson,[b] Mindy Meyers,[b] Dawit Tesfaye,[a] Fiona Hollinshead[b]

[a]Department of Biomedical Sciences,
[b]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA

Oviduct has a crucial role in gamete maturation, transport, fertilization, and early cleavage of embryos in the bovine female reproductive system. However, its anatomical positioning complicates physiological study. We aimed to develop an in vitro three-dimensional (3D) cell culture system replicating oviductal physiology to advance reproductive research. We established a 3D bovine oviductal organoid model, exposed to estradiol and progesterone at physiological concentrations to simulate estrus (2 days) and diestrus (4 days), creating a natural oviductal cellular microenvironment. Using single-cell transcriptomic profiling, we analyzed molecular responses of key cell types (mesothelial cells, secretory and ciliated epithelial cells) to hormonal stimulation. Cluster analysis revealed significant changes in the relative abundance of cell populations, with progenitor cells differentiating into more specialized epithelial secretory and ciliated cells. Single- cell RNA sequencing identified 3,106 differentially expressed genes (DEGs) between hormone- treated and vehicle-treated control groups. Among these DEGs, 3,074 genes were downregulated, whereas 27 were upregulated in hormone-treated organoids. Pathway enrichment analysis indicated significant suppression of metabolic and translational activities, particularly involving oxidative phosphorylation and ribosome-related processes in hormone-treated organoids compared to vehicle controls. These results provided valuable insights into bovine oviductal organoids that maintain a diverse array of cell types like those in vivo. Furthermore, the cell type- specific DEGs identified in the 2 treatment groups provided a comprehensive list of potential genes and pathways linked to the cell type-specific response to hormones and the physiological functions of different cell types in oviduct-embryo interactions. Bovine oviductal organoids serve as a promising model for studying oviductal biology and offer applications for enhancing reproductive technologies. Future studies will explore coculturing hormone-treated organoids with gametes or embryos or supplementing culture media with organoid-derived secretions, such as extracellular vesicles, to improve embryo viability and pregnancy outcomes.

Keywords: Cattle, oviduct, organoids, single-cell sequencing

Concurrent cystic endometrial hyperplasia, leiomyosarcoma, and pituitary adenoma in a goat

Noah Ennis,[a] Fred Williams III,[b] Elimar Ruiz Cortes,[c] Celeste Morris[c]

[a]College of Veterinary Medicine,
[b]Veterinary Medical Diagnostic Laboratory,
[c]Department of Food Animal Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA

A 12-year, female intact Nigerian Dwarf, goat was presented for aggression and mammary development of 1.5-2 years duration not responsive to GnRH and dinoprost tromethamine treatment. Goat had pale/tacky mucous membranes, rough hair coat, body condition score of 2/5, and soft bilaterally symmetrical mammary enlargement. Normal urine was passed and there was no appreciable vulvar/vaginal discharge; rest of the physical examination was within normal limits. Laboratory testing revealed a stress leukogram, mild anemia, and hypoproteinemia. Transabdominal ultrasonography revealed mixed echogenicity, disorganized structure in uterus and asymmetrical ovarian structures. Computerized tomography revealed suspect uterine mass with multiple intralesional cystic changes that compressed the bladder ventrally and to the left. Humane euthanasia was elected due to the presumptive neoplasia, chronicity of disease, and poor prognosis. Gross necropsy findings included a 2.5 cm mass at the level of the cervix that contained clear fluid and purulent exudate that extended into the uterus. There were also multiple 2-3 mm tan/pink nodules and 4-5 mm cysts grossly evident in the uterine wall. Ovarian follicles ranged from 2-5 mm. Milk-like fluid was expressed from mammary glands and the pituitary gland was grossly enlarged (2-3 times normal). Microscopic findings revealed leiomyosarcoma, cystic endometrial hyperplasia, and acidophilic corticotropic pituitary adenoma. Galactorrhea has been documented in goats with pituitary tumors[1,2] likely due to increased circulating prolactin,[3,4] but the reports are scarce despite this being a well-documented condition in humans.[4] Leiomyosarcomas with and without cystic endometrial hyperplasia have been documented in goats,[2,5-7] but Nigerian Dwarf goats are largely underrepresented and concurrent pituitary adenoma, leiomyosarcoma, and cystic endometrial hyperplasia has not been reported to the authors' knowledge. Due to increasing numbers of pet goats and thus prolonged lifespan, an understanding of neoplasia in these patients is essential and frequency of diagnosis is likely to continue increasing in coming years.

Keywords: Neoplasia, mammary, pituitary, uterine, goat

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Impact of pituitary pars intermedia disorder on the equine endometrium

Carleigh Fedorka,[a] Jocelyn Howard,[a] Isabella Hamner,[a] Rebecca Crook,[a] Brody Klinglesmith,[b] Stephen Coleman,[a] Patrick McCue[b]

[a]Department of Animal Sciences,
[b]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA

Pituitary pars intermedia dysfunction (PPID) is an age-related endocrinopathy associated with elevated adrenocorticotropin hormone (ACTH) and cortisol. Animals experiencing PPID have had elevated systemic inflammation and specifically an upregulation of the proinflammatory chemokine interleukin-8 (IL-8). It is unknown if this chronic inflammation, or the presumed fibrosis related to it, is noted within the reproductive tract. Therefore, the objective of this study was to evaluate the impact of PPID on the endometrium of the mare. We hypothesized that elevated ACTH leads to altered cytokine expression and increased fibrosis in the mare, predisposing them to endometritis. Aged mares (n = 11) were screened for PPID using a thyrotropin-releasing hormone (TRH) stimulation test in late summer. In brief, ACTH concentrations were measured before and 1-hour after intravenous treatment of 1.0 mg TRH. Of these, 7 mares were PPID positive (n = 7; ACTH > 110 pg/ml after stimulation), and 4 were PPID negative (n = 4; ACTH < 30 pg/ml after stimulation). When in diestrus (presence of a functional corpus luteum, increased uterine tone), 2 endometrial biopsies were obtained from all mares for qPCR analysis of select targets associated with inflammation (IL-8, IL-6, IL-1β, IFNγ), fibrosis (MMP2, MMP9, TIMP-2, and TNF) in addition to histology for fibrotic changes. Data were analyzed using SAS 9.4, and were assessed for normality and equal variances utilizing a Bartlett's and Shapiro-Wilk test. The impact of PPID on expression of transcripts relating to inflammation and fibrosis were evaluated using an unequal variances Student's t-test. Correlations between concentrations of ACTH and expression of transcripts were assessed using a Pearson correlation. Significance was set to $p < 0.05$. Of the targets evaluated, only IL-8 increased in expression in the PPID population ($p = 0.02$). There was a positive correlation between ACTH after TRH stimulation and the endometrial expression of IL-8 ($r = 0.80$; $p < 0.001$). A weak but significant correlation was also noted between ACTH and endometrial expression of IL-6 ($r = 0.41$; $p < 0.04$) and IFNγ ($r = 0.63$; $p < 0.01$). There was no significant correlation between concentrations of ACTH and expression of fibrotic markers. Additionally, no significant differences were noted when assessing fibrosis based on histopathology, as fibrotic changes were noted in 4 out of 7 PPID mares and 2 out of 4 control mares ($p = 0.82$). In conclusion, the systemic inflammation previously indicated in the PPID animal was also observed within the endometrium, but this was not associated with increased fibrosis. Future research is warranted to determine if this increase in IL-8 is associated with cytologic inflammation or active infection within the uterine lumen that would be detrimental to the fertility of PPID mares.

Keywords: Pituitary pars intermedia dysfunction, mare, endometrium, inflammation, endometritis

Association between endometrial swab bacteriology and cytology and live foal rates in Thoroughbred broodmares in the United Kingdom

Billy Fehin,[a] Camilla Scott,[a] Juan Arango-Sabogal,[b,c] Polly Rathbone,[c] Amanda de Mestre,[d] Rebecca Mouncey[c]

[a]Rossdales Veterinary Surgeons, Beaufort Cottage Stables, Newmarket, Suffolk, United Kingdom
[b]Département de Pathologie et Microbiologie, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada
[c]Department of Pathobiology and Population Sciences, The Royal Veterinary College, University of London, Hertfordshire, United Kingdom
[d]Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Relationships between prebreeding endometrial swab cytology and bacteriology and fertility outcomes in Thoroughbred broodmares in the United Kingdom (UK) have not been evaluated. Aims of this study were to investigate associations between cytology and bacteriology findings from the last endometrial swab taken in the breeding season and live foal rates (predicted mean probability of producing a live foal) in UK Thoroughbreds. We hypothesized that mares with a positive cytology and/or a positive bacterial culture is associated with lower live foal rates. Endometrial cytology and bacteriology findings from the last swabs taken in the breeding season (15th February-15th July) were collected from a database of all Thoroughbred endometrial swab samples submitted to Rossdales Laboratories between 2014 and 2020. Mares' status, age, and foaling outcome for each season were collected, where available, from publicly available data sources. Using a multivariable logistic regression model with mare and farm fitted as random effects, live foal rates were estimated for reported categories of cytology and bacteriology findings while adjusting for mares' age, status, number of previous swabs submitted in that season, and any interactions. Between-category rate differences within predictor variables and interaction terms were evaluated using pairwise comparisons with Bonferroni correction (statistical significance was

set at $p < 0.05$). Data were available for 7,691 last swabs from 3,579 mares on 196 farms. Mares with a profuse growth of *Escherichia coli* (*E. coli*) had significantly lower live foal rates (59.1%; 95% confidence interval (CI) 43.7-74.5) compared to those with no growth (80.9%; 95% CI 79.2-82.6). Live foal rates of mares with a profuse growth of β-hemolytic *Streptococcus* were not significantly different (76.6%; 95% CI 66.5-86.6) compared to those with no growth. There was interaction between mares' age and cytology findings. In mares over 12 years, significant reductions in live foal rates ($p < 0.05$ in pairwise comparisons) were observed between mares with > 30% polymorphonuclear (PMN): endometrial cells/high power field at cytological examination and mares with ≤ 0.5% PMN. Additionally, the predicted live foal rate was significantly higher in rested (89.4%; 95% CI 83.7-94.9) and barren (85.3%; 95% CI 82.9-87.7) mares compared to foaling mares (79.0%; 95% CI 77.1-80.9). The predicted live foal rate was significantly lower in mares that had either 2 (71.0%; 95% CI 67.2-74.8) or 3-10 (53.5%; 95% CI 48.1-59.0) previous endometrial swabs during the season compared to mares having none (85.0%; 95% CI 8.2-86.7) or 1 (83.2%; 95% CI 81.1-85.4) previous endometrial swab sample submitted. Results of the study highlighted complexities to consider when interpreting endometrial swab cytology findings and identified a subset of mares with a profuse growth of *E. coli* in which important knowledge gaps exist around etiologies underlying their poorer fertility outcomes. Careful attention regarding the reproductive management around breeding in mares over 12 years with evidence of a marked endometrial inflammatory response is warranted. Additionally, contrary to previous understanding, barren mares and rested mares were not associated with lower live foal rates in this study, suggesting that resting mares may be of benefit in terms of subsequent live foal rates.

Keywords: Horse, endometrium, cytology, bacteriology, live foal rates

Colloidal silver effects on semen parameters in dogs

Morgan Flanders, Fabio Pinaffi

College of Veterinary Medicine, University of Missouri, Columbia, MO, USA

Colloidal silver is used for therapeutic agents and drug delivery systems due to its antimicrobial and anti-inflammatory properties. However, toxic effects of colloidal silver have are known on male reproductive function in mammals.[1-3] To our knowledge, this is the first report on colloidal silver effect on canine sperm parameters. A 3-year, male intact Great Dane dog, with previously normal semen characteristics and fertility was presented with infertility and substantial abnormal sperm motility and morphology. No significant changes on libido or ejaculation were noted. Prior to the visit, the owner reported that the dog had injured his flank and developed dermatitis. Owner also reported treatment using antibiotics followed by topical treatment with colloidal silver-based ointment for several weeks. Dog was apparently healthy on physical examination and palpation of scrotal contents was unremarkable. Semen collection was performed by digital stimulation of the bulb of the penis, protrusion to the level of the bulbus glandis, and retroflexion to simulate the copulatory tie in the presence of a teaser female. Semen analysis was performed using a phase contrast microscope for sperm motility evaluation, NucleoCounter SP-100 (ChemoMetec) for sperm concentration measurements, and eosin-nigrosin stain under 1,000 x magnification light microscopy for sperm morphology evaluation. Two semen collections, performed 4 days apart, revealed a total of 1-1.5 x 10^9 sperm within each ejaculate, 5-10% progressive sperm motility, and 4-10% morphologically normal sperm, with detached heads accounting for 70-85% of the sperm morphological abnormalities. During this visit, the dog had been on topical colloidal silver for several weeks and possibly ingesting the ointment by licking that increased suspicions of colloidal silver toxicity. Therefore, we recommended the discontinuation of the colloidal silver ointment cream and performance of another semen analysis not < 2 months after discontinuation. Semen collection 5 months after discontinuation had considerable improvement in semen characteristics represented by a total of 556 x 10^6 sperm within the ejaculate, 70% progressive sperm motility, 80% morphologically normal sperm, and only 3% of sperm with detached heads. Colloidal silver caused considerable changes in sperm characteristics in dogs but effects were reversible within 5 months after discontinuation.

Keywords: Dog, colloidal silver, semen, morphology, detached head sperm

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Delayed embryonic development or long sperm survival in an embryo donor mare

Javier Funes, Jenny Sones, Patrick McCue

Equine Reproduction Laboratory, Clinical Sciences, Colorado State University, CO, USA

An embryo collection procedure is usually performed 7-8 days after ovulation in mares. Embryos are subsequently transferred into a synchronized recipient mare that is expected to carry the pregnancy to term. Submission of a blood or hair root sample to an approved genetics laboratory for DNA parentage verification is a prerequisite for foal registration for most horse breed organizations. The goal of this report is to document a clinical case in which the DNA parentage test of the foal produced by embryo transfer excluded the stallion whose semen was used on the estrous cycle yielding the embryo and identified as the genetic sire the stallion whose semen was utilized on the previous estrous cycle. A 21-year, Arabian mare, was inseminated with frozen- thawed semen from a deceased stallion (Stallion A) immediately after detection of a single ovulation. No embryo was

Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723

recovered following uterine lavage after 8 days. Cooled-transported semen from a different stallion (Stallion B) was used on the subsequent cycle. An exceptionally large (2,466 µm in diameter) expanded blastocyst stage embryo was recovered 8 days after ovulation and transferred into a recipient mare. The recipient mare carried the pregnancy to term. Genetic testing of the foal excluded Stallion B as the sire and confirmed Stallion A as the genetic sire of the foal. The potential explanations for how an equine embryo could be recovered following an insemination 26 days earlier include marked delayed fertilization or marked delayed embryonic development. Equine DNA parentage testing relies on the principle of exclusion, with the inheritance of a series of short tandem repeat (STR or microsatellite) markers evaluated in the foal, sire, and embryo donor mare. Parentage assignment based solely on breeding records would have been incorrect.

Keywords: Horse, fertilization, embryonic development

Geographical differences in prevalence and antimicrobial resistance of *Escherichia coli* isolates from dogs with pyometra

Isabella Gange,[a§] **Bianca Frehner,**[b§] **Julie Cecere,**[c] **Hannah Elshafie,**[a] **Sebastian Arlt,**[b] **Orsolya Balogh**[c]

[a]Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA, USA
[b]Department of Small Animal Reproduction, Vetsuisse Faculty, Zurich, Switzerland
[c]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA
[§]equal contribution

Pyometra is a common reproductive disease that affects intact female dogs. Treatment typically includes ovariohysterectomy or conservative medical management. Antimicrobial resistance is increasing in both human and veterinary medicine. We hypothesized that there are geographical differences in the prevalence and antimicrobial resistance profile of *Escherichia coli* (*E. coli*) isolates. Our aim was to compare the prevalence and resistance of E. coli to 4 commonly used antibiotics (amoxicillin-clavulanic acid (AMCLA), cefpodoxime (CEF), enrofloxacin (ENRO), and trimethoprim-sulfamethoxazole (TMS)) between a veterinary teaching hospital in the United States (H1) and Switzerland (H2). Medical records of dogs diagnosed with pyometra between 2010 and 2024 (H1:2010-2023, 109 dogs; H2:2017-2024, 122 dogs) were analyzed. Chi-square or Fischer's exact test with $p < 0.05$ for significance were used. Of the dogs where a bacterial culture was submitted, *E. coli* was in 60.3% (35/58, H1) and 65.6% (80/122, H2) of the patients ($p = 0.495$). *E. coli* was in pure culture in 91.4% (32/35, H1) and 87.5% (70/80, H2) of those cases ($p = 0.751$). When comparing all *E. coli* isolates with available sensitivity testing for a given antimicrobial between H1 and H2, 82.9 and 11.2% were resistant to AMCLA ($p < 0.001$), 22.5 and 6.1% were resistant to CEF ($p = 0.012$), 9.5 and 4.1% were resistant to ENRO ($p = 0.241$), and 10.0 and 11.2% were resistant to TMS ($p = 1.00$), respectively. Although there was no complete overlap in the data selection periods between the 2 hospitals, the preliminary results revealed a similar prevalence but different antimicrobial profile of *E. coli* isolates between 2 geographical regions. Due to the retrospective nature of this study, genomic analysis was not available. Our results highlighted the need for individual, well-considered, susceptibility test based and prudent antimicrobial use under consideration of geographical aspects.

Keywords: Antibiotic resistance, *Escherichia coli*, pyometra, uterus, infection, antimicrobial

Bilateral uterine horn segmental aplasia in a female goat

Adriana Garzon,[a] **Tessa Kell,**[b] **Bret McNabb,**[a,c] **Melissa Macias-Rioseco,**[d,e] **Kevin Keel**[b,d]

[a]William R. Pritchard Veterinary Medical Teaching Hospital, Livestock Herd Health and Reproduction Service,
[b]William R. Pritchard Veterinary Medical Teaching Hospital, Anatomic Pathology Service,
[c]Department of Population Health and Reproduction,
[d]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA, USA
[e]California Animal Health and Food Safety Lab, University of California, Tulare, CA, USA

In female embryos, the müllerian, or paramesonephric, ducts will develop into uterus, uterine horns, cervix, and the cranial third of the vagina. Failure in the development of the paramesonephric ducts during embryogenesis results in the absence of 1 or several segments of the uterus, known as segmental aplasia, and it has been described in multiple species, including goats and other ruminants. This report documents a case of bilateral segmental aplasia with secondary hydrometra and hydrosalpinx in a 2-year, nulliparous, Nigerian dwarf doe that was evaluated for failure to conceive. The doe had a normal vaginal and external cervical exam. Transabdominal ultrasonography revealed dilated anechoic fluid-filled, thin-walled segments of both uterine horns with a narrower tubular, fluid-filled structure that could be followed to the ovary; more caudally, there appeared to be normal sections of the uterus. The doe was given intramuscular cloprostenol (250 µg Estrumate®, Merck) twice, 10 days apart. After treatment there was no change in ultrasonography. The doe was euthanized due to a poor prognosis for reproductive success. Postmortem findings confirmed severe multifocal bilateral segmental uterine horn aplasia with hydrometra and hydrosalpinx. Bilaterally, several segments of the uterine horns were absent (uterine segmental aplasia). In place of the missing segments of the uterine horns, there was a thin, tan, firm fascial tissue. The distal uterine horns were markedly distended with clear, watery fluid (hydrometra). The true prevalence of uterine segmental aplasia in goats is unknown. Still, it should be included as a differential diagnosis for female infertility and as a cause of hydrometra in goats. Further research is warranted to better understand the prevalence and epidemiology associated with the etiology as well as possible genetic components of this condition in the goat.

Keywords: Segmental aplasia, goat, transabdominal ultrasonography, infertility

Assessing the impact of dog breeder mentorship and experiential learning on student knowledge and attitudes toward dog breeding

Meera Gatlin,[a] **Peter Nadel,**[b] **Ryan Viega**[b]

[a]Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA
[b]Tufts Research Technology, Tufts University, Somerville, MA, USA

The challenges of adequate knowledge and effective communication between veterinarians and dog breeder clientele are recognized,[1] yet theriogenology training is limited in veterinary curriculums,[2] requiring innovative educational tools to overcome these hurdles. Veterinary students at the Cummings School of Veterinary Medicine at Tufts University can enroll in a semester-long elective to learn about purebred dogs, breeding, and whelping directly through local breeder mentorship and experiential learning outside the classroom. A mixed-methods study was designed to determine the effectiveness of this mentorship and experiential learning on veterinary student knowledge and attitudes toward dog breeding and the dog breeder community. We hypothesized that experience improves veterinary student self-perceived knowledge on canine theriogenology topics and changes attitudes toward dog breeding and whelping. Identical pre and postcourse surveys were given at the start and end of the semester, respectively, to assess student knowledge and attitudes. The survey was comprised of 12 Likert scale questions about student perceived knowledge of canine theriogenology topics, 5 Likert scale questions about student perceived attitudes to breeder-veterinarian relationships and intentions to practice, and 4 free response questions about student perceived attitudes toward breeders and the breeding community. Survey responses were paired and Likert scale question comparisons were analyzed using Wilcoxon signranked tests in Python, with significance set at $p \leq 0.05$. Free response questions were analyzed using sentiment analysis, a natural language processing method used to detect emotional polarity and explain social phenomena. An increase was observed in all 12 knowledge topic areas as well as attitudes toward breeder-veterinarian relationships, the need for purebred and purpose-bred dogs, and confidence to practice with breeder clientele after graduation. Mean differences before and after mentorship had a general increase in positive sentiment toward questions about dog breeders, purpose-bred dogs, dog shows, and topics of interest in canine theriogenology. Students initially reported a lack of knowledge or experience with breeders and the breeding community but following the elective, acknowledged a positive change in attitudes and assumptions and referenced experiential learning as a productive tool. Canine breeder mentorship had a positive impact on veterinary student knowledge and attitude toward dog breeding. Biases and assumptions toward dog breeding and the breeder community were reframed following experiential learning. Study findings may be used to support dynamic extracurricular learning and strengthen the relationship among veterinary students, early-career veterinarians, and their future dog breeder clientele.

Keywords: Dog, breeding, reproduction, education, experiential learning

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Ultrastructural features and prostaglandin E secretion by equine trophoblastic vesicles

Josefina Ghersa,[a] **Marilyn Mullan-Fraser,**[a] **Nesma Yousif,**[a] **Daniel MacPhee,**[b] **Claire Card**[a]

[a]Department Large Animal Clinical Sciences,
[b]Department Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Maternal recognition of pregnancy (MRP) in mares remains unsolved, and trophoblastic vesicles (TRVs) may provide an in vitro means of studying this process. We hypothesized that TRVs secrete prostaglandin E (PGE) in a time-dependent manner in culture. Our aim was to characterize the ultrastructural features of TRVs derived from day (D) 14 equine embryos and to determine their ability to secrete PGE over time. Mares were bred with fresh semen from a known fertile stallion. Day 14 equine embryos (n = 3) were collected transcervically, washed with supplemented DMEM/F12 Hepes buffered media, the capsule was removed, and the trophoblast was cut into 2- 4 mm pieces. These pieces (n = 7 or 8) were cultured together in 500 µl DMEM/F12 media supplemented with 10% fetal bovine serum in a humidified incubator with 6% CO2, 5% O2, and 89% N2 at 37.5°C. Culture media (CM) was changed every 12 hours, pooled, filtered and stored at –80°C. Trophoblastic vesicles were maintained in culture for 4 days, then fixed, embedded, sectioned, stained with toluidine blue, and examined using transmission electron microscopy. In CM, PGE was measured using a PGE ELISA kit (Cayman Chemical) and protein concentration determined using a Bradford assay. Prostaglandin E concentration was standardized per mg of protein and normalized by log transformation. Differences in mean CM PGE/mg protein were assessed between embryos with one-way ANOVA at $p < 0.05$. TRVs from D14 embryos were round 1-2 cell layer structures with ultrastructural features compatible with trophoblast cells including microvilli, supranuclear vesicles and mitochondria, tight junctions, lipid droplets, multivesicular bodies, and active rough endoplasmic reticulum. TRVs spontaneously formed from embryo fragments, as early as after 12 hours of culture, grew, detached and multiplied for the first 48-60 hours, became static by 72 hours of culture, with some regression or deterioration at 84-96 hours. Median PGE concentrations in the CM peaked at 188 ng/mg protein at 24 hours of culture and then ranged 33-42 ng/mg protein through 96 hours. In conclusion, D14 derived TRVs shared similar ultrastructural and functional characteristics as the trophoblast of intact equine embryos and may provide an in vitro means to study aspects of the equine maternal recognition of pregnancy.

Keywords: Horse, trophoblast, vesicles, recognition, pregnancy, prostaglandin

Testicular neoplasm associated polyostotic hyperostosis in a male budgerigar

Alysa Giudici, Zoe Friel, Christopher Tollefson

Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

A 4-year, intact male budgerigar, was presented for evaluation of dyspnea. The bird had a chronic history of mild respiratory distress that progressed to increased respiratory effort, lethargy, and anorexia. Physical examination revealed a cere color change and caudal celomic mass. Oxygen therapy was initiated, and the bird was treated supportively for infectious and inflammatory etiologies with no improvement. Radiography revealed a dorsal solitary celomic mass adjacent to the left kidney, polyostotic medullary hyperostosis of the long bones, and possible increased opacity of the thoracic air sacs and lungs. The mass was prioritized as a testicular neoplasm, with seminoma or Sertoli cell tumor as top differentials.[1,2] Polyostotic medullary hyperostosis,[2] typically seen in female birds during ovulation, suggested a paraneoplastic syndrome associated with a testicular neoplasm. The bird was treated with a deslorelin implant to shrink the testicular mass,[3] but did not respond to treatment. Exploratory laparotomy confirmed a testicular mass and suspected metastasis throughout the celomic cavity. The patient died during surgery. Postmortem histology confirmed a metastatic sex cord tumor. This case highlighted the importance of reproductive neoplasms as a differential for respiratory distress in avian species, and utilization of imaging for early detection and diagnosis.

Keywords: Celom, mass, testicle, paraneoplastic, deslorelin, hyperostosis

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Effect of supplementation of donor mares with altrenogest on embryo recovery and size

Daniel Gomes, Patricia Xavier, Robyn Ellerbrock, Maria Ferrer

Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

Embryo transfer (ET) enhances genetic progress in sports horses and endangered breeds, yet the embryo recovery rate from donor mares remains as a substantial bottleneck. Given progesterone ìs critical role in embryonic support, this study hypothesized that altrenogest supplementation in fertile donor mares improves embryo recovery rates and size and optimizes the uterine environment. Objective was to evaluate the effect of altrenogest supplementation to donor mares on embryo recovery and size, corpus luteum size, and uterine environment. Five fertile donor mares were inseminated with fresh semen from the same fertile stallion over 2 estrous cycles using a crossover design. Control (CON) mares did not receive any exogenous hormones, whereas altrenogest (ALT) treatment mares received oral altrenogest supplementation (0.044 mg/kg daily between days 3-7 after ovulation). Embryo recovered on day 8 after ovulation via uterine lavage; embryo morphology and size, number and diameter of the corpus luteum (transrectal ultrasonography), and endometrial inflammatory cell count (endometrial cytology from the recovered fluid) were evaluated. Embryo recovery rate per cycle was 100% in ALT treatment and 83.3% in CON treatment ($p > 0.05$; Chi-square test). When calculated per ovulation, more embryos were recovered from ALT (100%) than CON (56%) treatments ($p = 0.042$; Chi-square test). Embryos from ALT treatments had larger median diameter (1135 µm; IQR 670-1140 µm) than embryos recovered from CON treatments (670 µm; IQR 560-840 µm) ($p = 0.134$; Kruskal Wallis test). Although not statistically different, this increase represented the recovery of ∼ 2 additional embryos per every 10 donor mares enrolled in an ET program when altrenogest was used. There was no difference in corpus luteum size (CON 26.8 ≤ 1.49 mm, ALT 28.98 ≤ 2.01 mm) (mean ≤ SD). No inflammatory cells were observed in the cytology of the fluid recovered from the uterus after embryo flush. In summary, embryo recovery rate increased by 20% per flush and 40% per ovulation in ALT-treated mares compared to controls, embryos were larger in ALT treatments. This study presented a novel application of altrenogest in mares, highlighting its potential for improving embryo recovery rates and size.

Keywords: Horse, embryo transfer, uterine environment, altrenogest

Effect of intrauterine ozone therapy on postbreeding inflammatory response in mares

Alexandra Grillos, Erin Runcan, Marco Coutinho da Silva

Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

Endometrial inflammation after breeding that lasts beyond 48 hours in the mare is classified as persistent breeding induced endometritis (PBIE). Proinflammatory cytokines (IL-1, IL-6, IL-8, IFNγ, and TNF-α) recruit white blood cells (WBC) to the uterus. Anti-inflammatory cytokines (IL- 4, IL10) work oppositely to modulate the immune response. Mares that are susceptible to PBIE have an increase in proinflammatory cytokines and a decrease in anti-inflammatory cytokines compared to normal mares within 6-24 hours after insemination. PBIE more commonly presents when breeding with frozen semen, due to the absence of seminal plasma that acts to dampen the immune response. Ozone is a gas molecule made up of 3 oxygen atoms in a cyclical structure, and was able to decrease inflammatory cells and microorganisms in the postbreeding uterine environment with fresh semen. As antimicrobial stewardship becomes more important while considering the development of new therapies,

ozone may have an important role as an alternative to antibiotic therapy. Our specific aims were to determine: 1. differences in the relative presence of WBC in the endometrium postbreeding in mares treated with intrauterine ozone or oxygen (control) and 2. differences in the endometrial mRNA expression of proinflammatory (IL-1, IL-6, IL-8, IFNγ, TNF-α) and anti-inflammatory (IL-4, IL-10) cytokines postbreeding. We hypothesized that treatment with intrauterine ozone 6 hours after breeding with frozen semen modulates the endometrial inflammatory response and reduces the rates of PBIE. Two healthy light-bred mares with negative endometrial culture and no signs of inflammation on endometrial cytology were used; mares were inseminated with frozen-thawed semen during estrus and treated with intrauterine oxygen (1.5 liter for 5 minutes) at 6 hours after breeding. An endometrial biopsy was collected at 24 hours after breeding and immediately frozen under liquid nitrogen. In addition, endometrial cytology was collected at 24, 48, and 72 hours after breeding. These mares then received a 21-day washout period, and the experiment was repeated with intrauterine ozone (50 µg/ml, 1.5 liter for 5 minutes). Percentage of WBCs in the cytology was compared by Fisher's exact test and data for gene expression were analyzed with a linear mixed effects model with significance set to $p < 0.05$. Endometrial cytology revealed only a slight decrease ($p > 0.05$) in the percentage of inflammatory cells present in the endometrium after ozone therapy (25.6%) compared to oxygen therapy (29.4%). In this preliminary experiment, endometrial biopsies were analyzed by RT-qPCR only for TNF-α. The relative changes in cytokine mRNA expression of TNF-α decreased ($p < 0.05$) in samples from mares that had been treated with ozone after breeding compared to oxygen.

Keywords: Horse, breeding, endometritis, ozone

Effect of cryoprotectant type at various steps of the cryopreservation process of stallion sperm

Camilo Hernández-Avilés, Jarred Crowley, Victor Zhidkov, Charles Love, Luisa Ramírez-Agámez

Equine Fertility Laboratory, Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Stallion sperm is cryopreserved in semen extenders formulated with penetrating cryoprotectants (CPAs), such as glycerol (G) and methyl formamide (MF). Some researchers have suggested that 'high' molecular weight CPAs such as G (92.1 g/mol) induce higher osmotic stress than 'low' molecular weight CPAs such as amides (e.g. MF 59.1 g/mol), resulting in higher oxidative stress and apoptosis in thawed sperm[1,2]; yet, experimental data demonstrating such an effect are still lacking. In this experiment, we measured the effect of adding G, MF or their combination (G + MF) to a 20% egg yolk-based (EY) freezing extender on stallion sperm quality parameters throughout the cryopreservation process. Ejaculates (n = 15) from sexually active stallions were collected and processed by cushioned centrifugation; sperm pellets were resuspended in a 20% EY freezing extender formulated with either 5% G, 5% MF, or 2% G + 3% MF (Step 1), cooled from 22 to 5°C at –1°C/minute (Step 2), cryopreserved to –196°C using an automated freezing system (–60°C/minute), and thawed at 37°C for 1 minute (Step 3). At each step, sperm motility was determined by CASA (% TMOT), whereas sperm viability (% Viab; SYBR-14/propidium iodide), apoptosis in viable sperm (% Apop-V; Yo-Pro-1/ethidium homodimer-1), and superoxide anion production in viable sperm (% SOX-V; MitoSOX red/SYBR-14/propidium iodide) were determined by flow cytometry. Data were rank-transformed for normalization and analyzed using the mixed-model ANOVA and Tukey-Kramer adjustment test (JMP Pro 17). Overall, within Steps, CPA effect ($p > 0.05$) was not observed. Mean TMOT, Viab, Apop-V, and SOX-V in fresh semen were (79, 79, 5, and 31%, respectively). In Step 1, mean TMOT, Viab, Apop-V, and SOX-V were similar among G (78, 78, 7, and 30%), MF (77, 76, 5, and 33%), and G + MF (79, 78, 6, and 32%; $p > 0.05$). In Step 2, mean TMOT, Viab, Apop-V, and SOX-V were similar among G (77, 76, 7, and 33%), MF (76, 75, 6, and 34%), and G + MF (77, 76, 6, and 35%; $p > 0.05$). In Step 3, mean TMOT, Viab, Apop-V, and SOX-V were similar among G (51, 53, 23, and 28%), MF (47, 47, 23, and 25%), and G + MF (49, 49, 23, and 28%; $p > 0.05$). When comparing the effect of each Step of the cryopreservation procedure on sperm quality, mean TMOT and Viab were similar in Steps 1 (73-76 and 75-76%), and 2 (71-73%, and 72-73%; $p > 0.05$), while higher than in Step 3 (47-51%, and 47-53%; $p < 0.05$). Mean Apop-V was similar in Steps 1 (5-7%) and 2 (6-7%; $p > 0.05$), while lower than in Step 3 (23%; $p < 0.05$). Mean SOX-V was similar across all Steps (25-34%; $p > 0.05$). In conclusion, cryopreservation of stallion sperm using either a 'high' (G) or 'low' (MF) molecular weight cryoprotectants, or their combination (G + MF) yielded similar sperm quality parameters throughout the sperm cryopreservation process.

Keywords: Stallion, sperm, cryopreservation, cryoprotectant, oxidative stress, apoptosis

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Factors affecting lactate-induced acrosomal exocytosis in viable frozen/thawed stallion sperm

Camilo Hernández-Avilés, Charles Love, Luisa Ramírez-Agámez

Equine Fertility Laboratory, Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Stallion sperm incubated under presumed capacitating conditions (presence of calcium, bicarbonate, and albumin) in a medium with lactate as the only energy substrate (Lac-MW) undergo protein tyrosine phosphorylation and spontaneous acrosomal exocytosis (AE) while retaining viability (AE/Viable).[1,2] In addition, by 4 and 6 hours of incubation in Lac-MW, the rate of AE/Viable in fresh and frozen/thawed stallion sperm are similar, and highly associated with the in vivo fertility of stallions.[2] In the current study, we determined some factors that may influence the occurrence of AE/Viable in frozen/thawed stallion sperm. In Experiment 1, to determine the effect of seminal plasma exposure to sperm, ejaculated (EJ) or epididymal (EP) sperm from 10 various stallions (n = 10) were cryopreserved. In Experiment 2, frozen/thawed sperm (n = 14 ejaculates, 7 stallions) were thawed and incubated in Lac-MW, Lac-MW with 50 mM added L- carnitine (Lac/Car-MW), or Lac-MW with 0.5 mM added penicillamine (Lac/Pen-MW). In both Experiments, after thawing, sperm were processed by density gradient centrifugation (40% silica particle solution), diluted to 30 x 10^6 sperm/ml in Lac-MW, and incubated for up to 6 hours at 38.2 °C in 5% CO2. At 0, 2, 4, and 6 hours of incubation, sperm aliquots were analyzed by flow cytometry for viability (% VIAB) and acrosomal status in viable sperm (AE/Viable). Data were rank-transformed for normalization and analyzed using the t-tests, the mixed linear model, and the Tukey-Kramer adjustment test. Statistical significance was set at $p < 0.05$. In Experiment 1, at all- time points, VIAB (0 hours: 52 versus 52%; 2 hours: 50 versus 53%; 4 hours: 49 versus 49%; 6 hours: 50 versus 50%, respectively) and AE/Viable (0 hours: 8 versus 6%; 2 hours: 16 versus 17%; 4 hours: 26 versus 30%; 6 hours: 38 versus 41%, respectively) were similar between EJ and EP groups ($p > 0.05$). In Experiment 2, at each point, VIAB (0 hours: 63 versus 63 versus 58%; 2 hours: 63 versus 55 versus 60%; 4 hours: 58 versus 59 versus 51%; 6 hours: 50 versus 51 versus 53%, respectively) was similar among Lac-MW, Lac/Car-MW, and Lac/Pen-MW groups ($p > 0.05$). At 0 hours (6 versus 5 versus 4%), 2 hours (21 versus 13 versus 26%) and 4 hours (33 versus 20 versus 23%), mean AE/Viable was similar among Lac-MW, Lac/Car-MW, and Lac/Pen-MW groups ($p > 0.05$); whereas at 6 hours, mean AE/Viable was higher ($p < 0.05$) in Lac-MW than in Lac/Car-MW (42 versus 26%;) and similar ($p > 0.05$) between Lac-MW and Lac/Pen-MW (42 versus 40%). Results indicated that lack of exposure to seminal plasma did not negatively affect the ability of epididymal stallion sperm to undergo lactate-induced AE in viable sperm. Also, adding 0.5 mM penicillamine, a thiol and antioxidant compound recently included in the formulation of a medium for conventional IVF in horses,[3] did not negatively affect the occurrence of lactate-induced AE in frozen/thawed stallion sperm. Current studies are focused on utilizing the Lac-MW model to achieve conventional IVF of in vitro-matured equine oocytes using frozen/thawed stallion sperm.

Keywords: Stallion sperm, acrosomal exocytosis, frozen/thawed semen, lactate, seminal plasma, penicillamine

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Congenital encephalocele in a live Friesian foal

Haylee Hutchins, Daniela Cortes, Rachel Pfeifle, Marc Kent, Robyn Ellerbrock, Maria Ferrer

Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

A 6-year, Friesian mare, was presented for foal watch at 335 days of pregnancy. Pregnancy was confirmed on day 30; transrectal ultrasonography at 4 and 8 months identified a fetus with normal heart rate, fetal fluids, and fetal membranes. On due date (April 15, 2024) the mare had fully developed udder that was streaming milk, with a pH of 6.3, predicting parturition within 72 hours. Fetal heart rate detection was challenging, ranging from 100-120 beats per minute. Mare foaled on day 339 of pregnancy with minimal assistance. Fetus was delivered 13 minutes after chorioallantois rupture in anterior longitudinal presentation, dorsosacral position, with extended legs and neck, but was not breathing. Mare retained fetal membranes that were removed 4 hours after parturition. Filly was unresponsive, displaying abnormal mentation, absent suckle reflex, focal seizures, anisocoria, suspected blindness, and an inability to attain sternal recumbency. During resuscitation attempts, a 1.5 × 4 cm skull defect with a 7.6 × 6.4 × 1.5 cm fluid-filled mass of brain tissue herniating through the frontal bone were identified. Additionally, there was left lateral thoracic scoliosis. Due to severe neurological impairment and congenital encephalocele, euthanasia was performed 5 hours after birth. Postmortem computerized tomography, magnetic resonance imaging, and necropsy confirmed encephalocele, a rare neural tube defect causing cerebral and meningeal herniation through the skull. Although typically congenital, encephaloceles can result from trauma, tumors, iatrogenic injury, or hypervitaminosis A. To our knowledge, this is the first documented case of a live foal born with an encephalocele. Signs of fetal stress were inconsistent, and the only concerns were a delay between the milk pH decrease and foaling as well as prolonged stage 3 of labor. This case highlighted the importance of detailed fetal ultrasonographic evaluation and stress monitoring for early identification of congenital malformations despite their poor prognosis.

Keywords: Encephalocele, fetal malformation, neural tube defect

Gangrenous mastitis in a dog after cesarian surgery

Bryson Jacobs, Joshua Trumble, Alexandra Grillos, Marco Coutinho da Silva, Erin Runcan

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

Mastitis is a disease diagnosed in postpartum lactating dogs where 1 or more mammary glands become infected by opportunistic pathogens.[1] This condition is not common in dogs compared to other species, and only encompasses about 5.3% of reproductive disorders in the dog.[2] A 4-year English bulldog dog was presented 4 weeks after cesarian surgery and ovariohysterectomy with anorexia and a swollen, bruised mammary gland. The referring veterinarian initially prescribed oral prednisone (0.5 mg/kg twice daily) and oral cephalexin (20 mg/kg twice daily), and pups were weaned. Examination of the mammary chain revealed an inflamed left cranial mammary gland with 2 open wounds, red discharge, and a 2-3 inch pocket of purulent fluid in the gland. Results from a complete blood cell count were consistent with an inflammatory/infectious process and a chemistry panel had no overt renal damage. Based on these findings, previous treatments were discontinued, and an alternative treatment plan was created due to minimal clinical response. Oral amoxicillin/clavulanate was empirically prescribed (15 mg/kg twice daily) pending culture and susceptibility results. Oral omeprazole (1 mg/kg twice daily) was also prescribed to prevent gastrointestinal ulceration from the prednisone. Following a 2-day prednisone withdrawal period, oral carprofen (2.2 mg/kg twice daily) was given. Culture results revealed heavy growth of *Escherichia coli* and *Enterococcus faecalis*, with resistance to amoxicillin/clavulanate and susceptibility to fluoroquinolones. Oral ciprofloxacin was then prescribed (10 mg/kg twice daily) and additional wound management with absorbent dressing was implemented as the mammary gland had begun to slough and drain. This case demonstrated the value of correct antimicrobial selection to successfully treat and manage gangrenous mastitis. It is important to swiftly diagnose and appropriately treat this disease as it can cause gangrenous necrosis of mammary tissue and become life-threatening if the animal becomes systemically septic.

Keywords: Dog, mastitis, postpartum, antimicrobial

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Comparative postbreeding outcomes in jennies inseminated with cryopreserved semen reextended in seminal plasma or treated with platelet-rich plasma

Carla Joseph, Theodore Blake, Claire Kaplan, Holly Schwarzman, Hilari French, Robert Gilbert, Lorenzo Segabinazzi

Ross University School of Veterinary Medicine, Basseterre, St. Kitts, West Indies

Artificial insemination (AI) with cryopreserved semen in donkeys is challenging due to poor fertility, likely caused by an exacerbated postbreeding inflammatory response. This study evaluated the effects of frozen-thawed semen reextension in seminal plasma (SP) and intrauterine infusion of platelet rich plasma (PRP) on postbreeding uterine inflammation, progesterone concentrations, and fertility in jennies. A total of 68 estrous cycles from 14 fertile jennies were randomly assigned to 1 of 5 groups: insemination with frozen-thawed semen reconstituted in 7 ml of SP (SP, n = 12); insemination with frozen-thawed semen followed by intrauterine infusion of lactated Ringer (Control, n = 14) or autologous PRP (PRP, n = 14) at 6 hours after AI; insemination with fresh semen (FS, n = 14); and a uninseminated group receiving PRP infusion at 44 hours after ovulation induction (PRP only, n = 14). Uterine lavage was performed 6 hours after AI in all cycles. Intrauterine fluid accumulation (IUF), endometrial neutrophil counts, corpus luteum (CL) volume, and plasma progesterone concentrations were assessed multiple times before and after AI. Pregnancy diagnosis was performed on day 14. FS and SP groups had lower neutrophil counts 6 hours after AI than Control and PRP groups ($p < 0.05$). PRP only cycles had the lowest neutrophil counts at 6 hours and 24 hours post AI ($p < 0.05$). Neutrophil counts were similar among all groups at 48 hours post AI ($p > 0.05$). Plasma progesterone was higher in FS cycles on days 3 and 8 compared to Control-assigned cycles ($p < 0.05$), and day 14 compared to all groups ($p < 0.05$). CL volume and IUF were similar across groups ($p > 0.05$). Pregnancy rates were higher in FS cycles (71%) compared to all other groups (Control, 0%; PRP, 14%; SP, 8%; $p < 0.05$). In conclusion, SP reduced postbreeding inflammation but did not improve fertility outcomes in jennies AI with cryopreserved semen, whereas PRP had no effect. Additionally, plasma progesterone was affected by the type of semen used for AI, but not by treatments.

Keywords: Donkey, frozen semen, PRP, endometritis, asinus

Megestrol acetate medication error induced diabetes mellitus in a cat

Audrey Kelleman,[a] Anum Ahmed,[b] Luis de Aguiar[a]

[a]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
[b]NorthStar VETS Veterinary Emergency, Trauma, and Specialty Centers, NJ, USA

A 2-year, Bengal cat (2.73 kg), was evaluated for suppression of estrus, due to cardiac abnormalities and risk of anesthesia for surgical sterilization. Additionally, the cat was to have a cardiology examination. Cat was the offspring of an accidental mating where 2 sibling female cats were produced that had congenital cardiac concerns. On auscultation, there was right sided systolic II/VI murmur; cat appeared clinically healthy. Cardiology examinations confirmed the murmur and identified a dilated aorta concurrent with right outflow obstruction. Various options were discussed with the client including oral progestogens and melatonin implant, but neither was available at examination. Oral megestrol acetate (0.95 mg/kg once weekly) was prescribed through a compounding pharmacy. A compounded solution of 40 mg/ml was prescribed with daily volume of 0.06 ml. Approximately after 2 months of therapy, cat was presented with the complaint of weight gain, ravenous appetite, and intermittent inappropriate urination. Estrus was suppressed. On interviewing the client, it was determined that the cat was given 0.67 ml of the compounded medication weekly, resulting in a tenfold medication error. Based upon clinical history, diabetes mellitus was suspected, and urinalysis of a free-catch specimen confirmed the diagnosis based upon presence of glucosuria. Urinalysis was otherwise unremarkable. Gradual reduction in medication volume was as follows: 0.3 ml once weekly for 2 weeks; 0.15 ml once weekly for 2 weeks; then 0.06 ml once weekly for suppression of estrus. Reduction was reportedly uneventful and follow up was directed with the regular veterinarian. The clinically normal cat returned the following year for examination, for refill of the product that had been utilized seasonally. Surgical sterilization was not performed to date.

Keywords: Cat, estrus suppression, progestogen, diabetes mellitus, glucosuria, murmur, cardiac anomaly

Effect of N-acetyl cysteine treatment on uterine cytokine and chemokine profiles in mares with persistent breeding-induced endometritis

Firdous Khan,[a,b] Michelle Caissie,[a] Bettina Wagner,[c] Elizabeth Scholtz,[a] Tracey Chenier[a]

[a]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada
[b]Department of Large Animal Medicine and Surgery, School of Veterinary Medicine, St. George's University, St. George's, Grenada
[c]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA.

Persistent breeding-induced endometritis (PBIE) is a major cause of infertility in mares; in PBIE- affected mares, treatment with intrauterine N-acetyl cysteine (NAC) 12-hours before breeding did not improve clinical signs at 12 and 60 hours after breeding.[1] The purpose of this study was to examine the effects of NAC treatment on the uterine inflammatory cytokine and chemokine profiles in PBIE-affected mares. Using a randomized, blinded, cross-over design, mares susceptible to PBIE (n = 10) were allocated to Control and Treatment cycles with at least 1 'washout' estrous cycle between the cycles. Intrauterine infusion of 180 ml of 3.3% NAC (Treatment) or sterile saline (Control) was performed 12 hours before insemination. Uterine fluid samples were collected at 12 and 60 hours after insemination to determine inflammatory cytokine and chemokine profiles. Endometrial biopsies were taken at the same time points to determine gene expression of selected inflammatory cytokines (interleukin-6, interleukin-10, interleukin-1β, and tumor necrosis factor-α). Differences between Control and Treatment cycles were analyzed for statistical significance using repeated measures ANOVA after removing data from a mare that failed 1 of the PBIE inclusion criteria. There was no difference ($p > 0.05$) in the uterine fluid inflammatory cytokines and chemokines between Control and Treatment cycles. Similarly, gene expression of the selected inflammatory cytokines did not differ between the 2 cycles. The absence of any significant effects of NAC on the uterine inflammatory cytokines and chemokines provided a potential mechanism to explain the previously reported lack of improvement in clinical signs in PBIE-affected mares treated with NAC.

Keywords: Horse, insemination, uterus, inflammation, N-acetyl cysteine

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Clinical management practices of equine endometritis in India

Afroza Khanam,[a] Aeknath Virendra,[b] Caitlin Coartney,[a] Giana Gigantino,[a] Alok Kumar,[c] Firdous Khan[a]

[a]St. George's University-School of Veterinary Medicine, St. George's, Grenada
[b]Maharashtra Animal and Fishery Sciences University, Mumbai, India
[c]Bihar Animal Sciences University, Patna, India

Endometritis is a major cause of infertility in mares. Limited information is available on the clinical management practices of equine endometritis in India. The objective of this cross-sectional study was to collect information from veterinarians in India on their diagnostic and treatment practices of equine endometritis. The information was collected using a 25 question survey sent electronically to veterinarians in India. Participation in the survey was voluntary, and all responses were collected anonymously. A total of 102 veterinarians from various states and union territories of India responded to the survey. The participants reported encountering acute infectious endometritis the most (41%), followed by persistent breeding-induced endometritis (29%). Most veterinarians

used uterine swabs (69%) and transrectal examinations (60%) for diagnosis of endometritis. Uterine biopsy was rarely used as a diagnostic tool (8%), likely due to its perceived negative impact on fertility (35%) or uncertainty about its effects on fertility (33%). Uterine bacterial culture often detected *Escherichia coli* (39%) and *Streptococcus equi* subspecies *zooepidemicus* (34%) associated with endometritis. Although most veterinarians reported using a combination of systemic and intrauterine antibiotics, there was a substantial number of respondents (24%) did not use culture and antimicrobial susceptibility to select antibiotics. This practice constitutes indiscriminate use of antibiotics and can worsen the already concerning antimicrobial resistance in the country. The results of this study suggested that there is a need for increased awareness and education on equine endometritis, more standardized diagnostic and treatment protocols, and more research on the disease in the Indian context.

Keywords: Horse, endometritis, diagnosis, treatment, antimicrobial resistance

Twin pregnancy in a southern tamandua (*Tamandua tetradactyla*)

Taylor King,[a] Mike Wenninger,[b] Jess Heinz,[b] Jenny Nollman,[b] Christopher Premanandan,[c] Julie Barnes[d]

[a]College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada
[b]Cincinnati Zoo & Botanical Garden, Cincinnati, OH, USA
[c]Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA
[d]Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH, USA

Southern tamandua is characterized by a simplex uterus, uniparous reproduction, and a discoid hemochorial placenta, with an average duration of 160 days (range; 130-190 days) of pregnancy. Although twins are possible in this species, they are rare and not well documented. Here, we report the only known case of a twin pregnancy with a live birth in the Association of Zoos and Aquariums population. To the authors' knowledge, there is only 1 other twin pregnancy observed in this population that resulted in abortion. A 10-year, multiparous female, chronically treated with methimazole for hyperthyroidism and previously documented with a 2.1 x 3.3 mm uterine cyst prior to pregnancy, was genetically valuable to the population and paired with a proven male for breeding. Twin pregnancy was diagnosed on day 117 prepartum (PP; 35-42 days after breeding) via voluntary ultrasonography. Fluid was observed around the heart of Fetus 1 (F1) on day 54 PP (98-105 days after breeding), and F1 died in utero 20 days after detection. Twice daily pentoxifylline (10 mg/kg) treatment began on day 46 PP to support the pregnancy through parturition. Fetus 2 (F2) was born alive and hairless between 152-159 days after breeding, but died 8 hours after birth, with no substantial lesions on histopathology. An intact placenta was recovered, having a single chorioallantois containing 2 distinct chambers and a separate blood supply for each fetus. There was an absence of fetal capillary structures and moderate multifocal interstitial hyalinosis of terminal chorionic villi in portions of the placenta that may have contributed to the death of F1. The dual-chambered placenta raised the question of monozygotic or dizygotic twins. DNA samples from both parents and offspring have been submitted to evaluate the genetic similarity between the twins.

Keywords: Southern tamandua, twin pregnancy, hemochorial, pentoxifylline, avascular villi

Changes in body condition score, trace minerals by parity, physiological state, and their influence on postpartum resumption of estrous cyclicity in beef cattle

Sai Kumar, Kamal Gabadage, Jose Alcivar de Lucca, Sarah Parker, Devinda Wickramasingha, Dinesh Dadarwal

Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Trace minerals (TM) are essential for immune function, reproductive health, and metabolic processes in beef cattle during the peripartum period, supporting the health of cow and calf. However, studies on the temporal variations of TM during late pregnancy and the postpartum period in beef cows are limited. This study aimed to investigate changes in peripheral and hepatic TM concentrations and examine the effects of body condition score (BCS) and parity on the resumption of estrous cyclicity in postpartum beef cows. We hypothesized that TM concentrations fluctuate to meet metabolic demands, vary by parity, and are associated with the resumption of estrous cyclicity in postpartum cows. Pregnant beef heifers (n = 22, 2 years) and cows (n = 26, 4-7 years, parity: 3-6) in their last trimester were enrolled. Blood and liver biopsy samples were collected during the prepartum (76 ± 20 days) and postpartum (42 ± 8.4 days) periods. BCS was assessed at each sampling time using a 9-point scale (1 = emaciated to 9 = obese). Samples were analyzed for magnesium (Mg), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo) using mass spectrometry. Additionally, all animals were evaluated for the resumption of estrous cyclicity 40 ± 3 days postpartum using transrectal ultrasonography. Changes (Δ) in BCS and TM concentrations from prepartum to postpartum stage were analyzed using the Wilcoxon signed-rank test. Differences in ΔTM concentrations between primiparous and multiparous cows were assessed using the Mann-Whitney U test. Estrous cyclicity data were analyzed using Fisher's exact test and logistic regression. The primiparous cows exhibited a higher reduction in median BCS than in multiparous cows (1 versus 0.5; $p < 0.001$). Overall, peripheral Co and Se concentrations decreased ($p < 0.001$) in postpartum compared to prepartum by 68 and 39%, respectively. Conversely, hepatic concentrations of Mg, Co, Se, and Mo decreased ($p < 0.01$) by 20, 42, 24, and 8%, respectively whereas Mn and Zn increased ($p < 0.01$) by 14 and 13%, respectively. Postpartum hepatic Mn and Cu concentrations increased ($p < 0.05$) in multiparous cows but decreased in primiparous cows. In contrast, hepatic Zn concentration was higher ($p < 0.001$) in primiparous cows than in multiparous cows. Postpartum multiparous cows exhibited a smaller reduction ($p < 0.001$) in hepatic Co (25.8 versus 33.7 ppm) and peripheral Co (0.7 versus 0.3 ppm) compared to primiparous cows. The ΔMo in liver was higher ($p < 0.001$) in multiparous cows than in primiparous cows (0.11 versus 0.02 ppm). Postpartum blood Cu concentrations decreased in multiparous cows but increased in primiparous cows

Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723

($p < 0.001$). Twenty-two out of 48 cows resumed estrous cyclicity at 40 ± 3 days postpartum. The proportion of cyclic cows was higher ($p < 0.001$) in multiparous cows than in primiparous cows (19/26 versus 3/22). A unit increase in ΔBCS decreased the odds of estrous cyclicity resumption by 82%. These findings supported our hypothesis that TM concentrations vary with physiological stage and parity, with liver TM concentrations having more pronounced changes than peripheral blood. These findings suggested the importance of TM in metabolic adaptations during the postpartum period in beef cows.

Keywords: Hepatic minerals, last trimester, liver biopsy, postpartum

Prenatal ultrasonographic diagnosis of kidney abnormality in an equine fetus

Nayara Laquiz, Sofia Kovacsy, Luis de Aguiar, Audrey Kelleman, Eduardo Arroyo, Maria Cadario

College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Prenatal ultrasonographic examination is a potent diagnostic tool frequently overlooked during mid-late pregnancy in the mare. The combination of transrectal and transabdominal examination evaluates the fetus and placenta, providing crucial information about pathological conditions. Aim of this report is to present the evolution of a case of congenital kidney abnormalities. An 18-year, Warmblood multiparous broodmare, was bred with frozen semen in July 2024. No abnormalities were detected on days 14, 25, 45, and 60 of pregnancy ultrasonographic examinations. At 120 days, in a follow-up examination, the fetal kidneys appeared hyperechogenic and dysplastic (length: 34 and 35 mm). Combined uteroplacental thickness (CUPT) was 4.5 mm and fetal parameters, including activity (grade 2 out of 3), heart rate (118 beats per minute), aortic diameter (5.5 mm), fluid depth, and echogenicity (dark grey), as well as the abdominal and thoracic organs, were within normal limits. Day 143 ultrasonographic images revealed widespread kidney cystic structures. Subsequent pregnancy evaluations had progressive enlargement of the renal cystic structures. Initial differential diagnosis included developmental renal cystic disease (DRCD), fetal kidney dysplasia, and congenital hydronephrosis. DRCD is a congenital or sporadic kidney malformation that occurs early in organogenesis due to abnormal differentiation of the metanephric duct system. The presentation has distinct forms, including aplastic, hypoplastic, obstructive, multicystic, and diffuse patterns. The most frequent forms are autosomal recessive polycystic kidney disease (ARPKD) observed perinatally or neonatally in dogs, cats, sheep, and horses, followed by autosomal dominant polycystic kidney disease (ADPKD), most observed in adult dogs and cats. In the event of fetal kidney dysplasia, it can present as abnormal renal size, structure (cysts), or function. The dysplastic changes during fetal development are dynamic, unilateral, or bilateral; therefore, continuous evaluation is advised. With respect to prenatal hydronephrosis, enlargement or dilation of the kidneys, specifically the renal pelvis, is very characteristic. It can occur unilaterally or bilaterally and is often caused by a blockage in the urinary tract or the reflux of urine from the bladder to the kidneys. Ultrasonography typically reveals lobulated kidneys with anechoic content, devoid of renal parenchyma. However, this pathology was ruled out on day 143 of pregnancy based on the ultrasonographic images that exhibited hyperechogenicity and cystic- like structures. Although the present case did not exhibit all the clinical manifestations of ARPKD, it was initially diagnosed as such. This disease typically manifests as bilateral enlargement of echogenic kidneys, accompanied by hepatic cystic development and dysfunction. Since the kidneys have a crucial role in fetal development, as the disease progresses, it compromises the growth of the fetus. Pulmonary hypoplasia can also occur due to oligohydramnios and the increased pressure in the chest cavity caused by the enlarged kidneys. Renal cystic disorders in equine are rare, and the genetic mutation responsible for the condition remains unknown. Consequently, fetal well-being, development, and uterine examination throughout pregnancy are essential for early diagnosis of congenital disorders. This case emphasized the importance of ultrasonography in detecting high risk pregnancies and the need for the development of genetic testing for horses.

Keywords: Congenital, horse, polycystic, fetal development, kidney

Unlocking the future of equine fetal sexing: mass spectrometry analysis of maternal conjugated estrogens in serum

Joy Ledeck,[a,b] Thomas Dubrowski,[b] Matthieu Schoumacher,[b] Sophie Egyptien,[a] Stefan Deleuze,[a] Caroline Le Goff,[b] Stéphanie Peeters,[b] Etienne Cavalier,[b] Jérôme Ponthier[a]

[a]Equine Clinical Sciences Department, FARAH Comparative Veterinary Medicine,
[b]Department of Clinical Chemistry, CIRM, CHU de Liège, University of Liège, Liège, Belgium

Since 1976, estrogen concentrations in pregnant mares have been thought to be unrelated to fetal sex, but these studies were based on immunoassays. Despite their widespread use, these assays have limited specificity due to cross-reactivity and are rarely validated in equine, compromising their accuracy. Liquid chromatography tandem mass spectrometry (LC-MS/MS) provides precise steroid quantification, allowing deeper insights into steroid metabolism and potential fetal gender differences. The current standard for fetal sexing, transrectal ultrasonography, requires technical expertise, has a limited gestational window, and may yield inconsistent results. This study hypothesizes influence of fetal sex on maternal sulfonated estrogen concentrations and aims to develop a noninvasive method for fetal gender determination in mares. From 2020-2024, 68 mares from Belgian stud farms, managed under standardized conditions regarding diet, housing, and reproductive practices, were included, resulting in 115 pregnancies. Blood samples (n = 596, median per pregnancy: 5 (Q1-Q3: 4-6) were collected from 4-11 months to quantify estrone-sulfate (E1S) and estradiol-sulfate (E2S) using a validated LC-MS/MS method for equine. Mares with placentitis were excluded. Data were analyzed using logistic regression (SAS 9.4, $p < 0.05$) to assess fetal sex effects, accounting for maternal age, breed, and parity, and estimate associations between fetal sex and maternal hormone concentrations. Most mares were Warmbloods (45.6%) or Spanish purebred horses (44.1%). Male foals accounted for 51.7% of the births, with a sex ratio of 1:1.07. Parity and breed did not affect the sex ratio, whereas age tended to be significant ($p = 0.06$). Estrone concentrations followed a quadratic trajectory ($p = 0.0003$), peaking at 5 months for females and 6 months for males. Fetal gender

influenced hormone concentrations for E2S ($p < 0.0001$) and E1S ($p = 0.012$). Males exhibited higher E2S from 169-308 days, with the most significant differences at 169-196 days ($p = 0.0003$), 197-224 days ($p = 0.0019$), and 225- 252 days ($p = 0.0031$). Females had higher E2S at 113–140 days ($p = 0.032$). These results contrast with previous reports, where no fetal sex-related differences in maternal estrogen concentrations were observed. Fetal gonads secrete androgens that drive placental estrogen production, and their bioavailability influences maternal estrogen concentrations. At 5 months, female fetuses reach peak E2S concentrations, exhibiting higher concentrations than males. Around 6 months, male fetuses surpass females in E2S concentrations as they reach their peak. This shift is due to differential steroidogenic activity. Although male fetal gonads contain fewer interstitial cells at this stage, their enzymatic machinery is more developed, leading to greater androgen production. These are aromatized in the placenta into estradiol by cytochrome P450 19A1, whose transcript expression also peaks around 6 months, before placental sulfotransferases responsible for estrogen sulfonation increase E2S excretion in maternal circulation, particularly with male fetuses. These findings suggested maternal E2S concentrations differ by fetal sex, indicating LC-MS/MS could serve as a reliable, noninvasive alternative to ultrasonography for fetal monitoring. Expanding the cohort earlier in pregnancy could improve predictive accuracy and enable earlier fetal sex determination via ultrasonography, optimizing breeding management.

Keywords: Mare, pregnancy, sulfonated estrogens, fetal sex, liquid chromatography tandem mass spectrometry

Effect of FSH and progesterone on canine cumulus-oocyte complexes metabolism

Jose Len,[a,b] Lara Madding,[b] Jacob Howard,[b] Jasmine McIver,[b] Chin-Chi Liu[a]

[a]Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
[b]Department of College of Veterinary Medicine, NC State University, Raleigh, NC, USA

Objective of the study was to assess the effect of supplementing the maturation medium with hormones on canine cumulus-oocyte complexes (COCs) metabolism during in vitro maturation (IVM). We hypothesized that medium supplemented with FSH or progesterone (P4) stimulates canine COCs metabolism, as measured by oxygen consumption rate (OCR) and proton efflux rate (PER), compared to medium without supplementation. Ovaries from dogs (> 1.5 years) were sliced in a petri dish (60 mm) containing medium 199 with Hanks' salts supplemented with 10% fetal calf serum (FCS) and 1% v/v penicillin/streptomycin. Groups of 10 COCs were randomly allocated into the following groups: 1. Control treatment group (CON); DMEM supplemented with 10% FCS, 1% Penicillin/Streptomycin, 1 µl/ml insulin-transferrin-selenium and 2.5 mM L-glutamine; 2. FSH treatment group: CON supplemented with 5 µg/ml of FSH; and 3. P4 treatment group: CON supplemented with 40 µg/ml of progesterone. The OCR and PER of COCs during IVM were measured every 24 hours using extracellular flux analysis (Seahorse XF Real-Time ATP Rate Assay Kit, Agilent Technologies). A repeated measure analysis of variance (ANOVA) with a mixed effect model was used ($p < 0.05$). Treatment, day and their interaction were entered in the model as the fixed effects, and each replicate was entered as the random effect. At 24 hours, OCR within and between CON (10.2 ± 0.8 pmol/minute/COC), FSH (10.8 ± 0.9 pmol/minute/COC) and P4 (11.5 ± 0.9 pmol/minute/COC) treatment groups were not different but higher than at 48 and 72 hours. PER within CON at 24 hours (5.7 ± 3.2 pmol/minute/COC) was lower than at 48 hours (22.4 ± 3.2 pmol/minute/COC) but not different than at 72 hours (14.3 ± 3.2 pmol/minute/COC). PER within FSH was not different at 24 hours (18.3 ± 3.3 pmol/minute/COC) and 48 hours (29.0 ± 3.3 pmol/minute/COC) but lower at 72 hours (16.5 ± 3.3 pmol/minute/COC). Within the P4 treatment group, PER at 24 hours (15.6 ± 3.4 pmol/minute/COC), 48 hours (14.9 ± 3.4 pmol/minute/COC) and 72 hours (17.0 ± 3.4 pmol/minute/COC) were not different. Among treatment groups, PER was not different at 24 hours, but at 48 hours PER of P_4 was lower. At 72 hours, there was no difference in PER among treatments. To authors' knowledge, this is the first report evaluating canine COCs metabolism during IVM using extracellular flux analysis. Supplementation of the maturation medium with either FSH or P_4 did not increase the metabolism of canine COCs and the hypothesis was rejected. Real-time measurement of metabolic rate (OCR and PER) revealed that COCs produced ATP by both oxidative phosphorylation and glycolysis during the first 24 hours of IVM. However, after 48 and 72 hours of IVM, ATP production shifted to the glycolytic pathway. In all treatments, OCR was < 4 pmol/minute/COC and < 3 pmol/minute/COC and PER was > 14 pmol/minute/COC and > 11 pmol/minute/COC, at 48 and 72 hours, respectively.

Keywords: Dog, oocyte, cumulus, metabolism, in vitro maturation

Validation of a portable computer assisted semen analysis system for evaluating progressive motility and concentration of stallion and bull sperm in field and laboratory settings

Georgia Lefaivre,[a] Jennifer Pearson,[a] Deanna Larsen,[b] Juan Hernandez-Medrano[a]

[a]Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada
[b]Fen Vet Equine Reproduction, Carstairs, AB, Canada

We evaluated a portable computer-assisted semen analysis (PC) system (AndroScope, Minitube) for assessment of progressive motility (PM) and concentration of stallion and bull sperm by comparing the system to reference laboratory and field-based methodologies. Stallion (n = 12) and bull (n = 13) semen samples were collected and diluted using commercial extenders (INRA96, IMV Technologies and Triladyl, Minitube, respectively) or fixed in phosphate buffered saline formalin solution. In the field, extended samples were analyzed for PM by light or phase-contrast microscopy and PC, and for concentration by PC. In the laboratory, extended samples were analyzed for PM by PC and a laboratory-based computer-assisted semen analysis (LC) system (SpermVision, Minitube) as well as for concentration with the PC. Formalin-fixed samples were analyzed for concentration using a hemocytometer. Correlation and agreement between PC and LC, field microscopy, and hemocytometer methodologies for PM and concentration were assessed by Spearman tests and Bland-Altman analyses, respectively. In stallions, PM measured by

Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723

PC strongly correlated with field microscopy (r = 0.71) and LC (r = 0.90). Conversely, PC underestimated PM by 11.9 ± 13.4% (mean ± SD) compared to field microscopy, and overestimated PM by 1.5 ± 8.4% when compared to the LC. Concentration measured by PC was strongly correlated with the hemocytometer (r = 0.83) but overestimated by 109 ± 64.1 x 10^6 sperm/ml. In bulls, PM measured by PC correlated fairly with field microscopy (r = 0.39) but strongly to LC (r = 0.91). However, PC underestimated PM by 7.9 ± 11.0% compared to field microscopy and overestimated by 6.5 ± 5.0% compared to LC. In bulls, PC revealed poor correlation (r = 0.11) and severely underestimated sperm concentration (-908.0 ± 619.0 x 10^6 sperm/ml). Overall, the PC adequately estimated PM, particularly in stallion semen, but overestimated sperm concentration in both species. In practice, care should be taken when interpretating the results from automated semen evaluation methodologies and consider further calibration of these systems to avoid over or underestimation of semen parameters.

Keywords: Semen concentration, progressive motility, CASA, stallion, bull

Effects of firocoxib on oocyte quality in mares undergoing repeated transvaginal ultrasound-guided follicular aspiration procedures

Victoria Lindsay,[a] Alex Wittorff,[a] Amber Hampton,[a] Carolina Okada,[b] Carlos Ramires Neto,[b] Kelli Beavers,[a] Dale Kelley,[a] Reed Holyoak,[a] Giovana Catandi[a]

[a]Department of Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA
[b]GeneTech USA, Purcell, OK, USA

Advanced reproductive techniques, such as transvaginal ultrasound-guided follicular aspiration (TVA) and intracytoplasmic sperm injection (ICSI), have seen remarkable growth in the equine industry. Many donor mares in TVA-ICSI programs are older and managed with nonsteroidal antiinflammatory drugs (NSAIDs) for chronic musculoskeletal conditions. A recent study identified a transient decline in oocyte quality and embryo development following TVA-ICSI of mares treated with oral phenylbutazone (4.4 mg/kg) once daily for 10 days. We hypothesized that using a COX 2 specific inhibitor (firocoxib) maintains or improves oocyte developmental competence in mares undergoing TVAs. Four mares (8.7 ± 2.1 years) underwent 5 TVAs at 14 day intervals. Oral firocoxib (0.1 mg/kg) was given once daily for 10 days before the fourth TVA. Collected cumulus oocyte complexes (COCs) were transported in a holding medium (EmCare, ICPbio), matured in vitro (38.2°C, 6.7% CO2, 5% O2), fertilized by ICSI using frozen-thawed semen from a single proven stallion, and cultured (38.2°C, 5.1% CO2, 5% O2) until blastocyst formation. Liquid nitrogen frozen follicular fluid and plasma were submitted for quantification of proinflammatory chemokines. Cumulus cell expansion rates, oocyte maturation, cleavage and blastocyst development were compared by chi-square tests. Comparisons for chemokine concentrations were completed using repeated-measures ANOVA or mixed effect analysis (significance $p < 0.05$). COC expansion rates were significantly higher after the fifth TVA (~ 2 weeks after firocoxib) than in previous TVAs. Although oocyte maturation, embryo cleavage and blastocyst rates did not reach statistical significance, they were numerically highest following firocoxib treatment (TVAs 4 and 5). No significant differences in chemokine concentrations in follicular fluid or plasma were observed. Ongoing analyses will evaluate gene expression of proinflammatory cytokines and enzymes in granulosa cells and firocoxib concentrations in plasma/follicular fluid. These preliminary findings suggested that firocoxib treatment did not negatively impact oocyte quality in mares undergoing TVA-ICSI.

Keywords: Mare, firocoxib, oocyte, maturation, transvaginal ultrasound-guided follicular aspiration

Effect of knockout serum replacement supplementation in culture medium on bovine blastocyst gene expression after cryopreservation

Erika Mackenzie,[a] Hugo Hernandez-Fonseca,[a] Andy Alhassan,[b] Bhumika Sharma,[b] Fernando Perea,[c] Hector Zerpa Gonzalez,[a] Diana Gabriela Peña,[d] Sofia Belén Vera,[d] Daniel Arqudo[d]

[a]Department of Animal Anatomy, Physiology, & Pharmacology,
[b]Department of Veterinary Pathobiology, School of Veterinary Medicine, St. George's University, Grenada, West Indies
[c]College of Agricultural Sciences, University of Cuenca, Cuenca, Ecuador
[d]Group for Reproduction in Animals, Vaccinology and Infectious Diseases, Department of Population of Heath, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA

Embryos used for bovine in vitro fertilization (IVF) are cryopreserved to protect cell morphology, maintain proper gene expression regulators, and increase longevity. Early embryonic mortality is a major cause of reproductive failure in many species. The medium in which the embryos are cryopreserved can influence the success of embryo survival after preservation. This study investigated the effect of a Knockout Serum Replacement (KSR; Thermofisher) and the connection between preservation medium and embryo survival and competence after cryopreservation. Blastocyst supplemented with KSR were expected to have a greater expression of genes involved in embryo survival compared to those that did not after cryopreservation. Embryos were cultured for 6 days in a medium supplemented with 1 of 3 treatments: 5% KSR, 5% fetal bovine serum (FBS), or 0.6% bovine serum albumin (CON). On day 7, after fertilization, embryos were classified based on their morphology. Quality 1 blastocysts were selected to be frozen, stored and then thawed to test genes associated with growth and development of blastocele re-expansion after thawing identified as aquaporin 3 (AQP3), sphingosine-1-phosphate phosphatase 1 (SGPP1), Bcl-2-associated X-protein (BAX), and glyceraldehyde 3-phosphate-dehydrogenase (GAPDH; housekeeping gene) via real time polymerase chain reaction. Percentage of reexpansion of thawed blastocysts was greater in the CON groups than in the KSR or FBS groups. No significant differences were observed in any of the tested genes among the knockout treatments. Under the described conditions, KSR did not increase the expression of genes associated with embryo survival nor did it increase the survival of embryos after cryopreservation.

Keywords: Embryos, cattle, cryopreservation, blastocyst, KSR, in vitro fertilization,

Associations between dismount semen evaluation, postmating antibiotics, and mare clinical parameters

Humberto Magalhaes, Igor Canisso

Department of Veterinary Medicine, University of Illinois at Urbana Champaign, Urbana, IL, USA

Hand mating is the only acceptable method of breeding in Thoroughbred mares. Confirmation of ejaculation and monitoring of semen quality is performed via dismount samples collected from the mare's cranial vagina or more typically, placing the glans penis in a receptacle as the stallion is coming off the mare's back after mating. Also, hand mating poses substantial challenges (e.g. transmission of venereal and infectious diseases). Strategies to minimize such issues include screening for infectious diseases and postmating uterine infusion of antibiotics such as ceftiofur or ticarcillin. This study aimed to determine the associations between dismount sample features and mare clinical parameters during hand mating. The study involved 50 matings of 25 mares and 3 fertile stallions. Ovulation was induced with a GnRH-agonist (histrelin acetate, Wickliffe), and mares were mated 24 hours later under the maiden mare sedation protocol (intravenous xylazine (100 mg), butorphanol (10 mg), acepromazine (20 mg)). Uterine cultures and cytology were performed 4 hours, and 3 and 5 days after mating. After mating, the dismount semen sample parameters were assessed for volume and concentration; semen kinetics, such as motility, were evaluated using a portable sperm analyzer (iSperm). Mares underwent daily transrectal ultrasonography for 6 days after mating to confirm ovulation and detect uterine fluid. Uterine infusions were performed 4 hours after mating and then daily for 5 days immediately after each ultrasonography. Each mare underwent a control cycle (60 ml lactated Ringers solution (LRS)/infusion, 25 cycles) followed by an antibiotic cycle (ceftiofur reconstituted in distilled water 20 ml with 40 ml of LRS, n = 15 cycles or 3.1 of ticarcillin-clavulanate reconstituted in distilled water 20 ml + 40 ml of LRS, n = 10 cycles) for 5 infusions. No other postmating therapies were applied except for uterine infusions. Pregnancy was confirmed 14 days after ovulation and then terminated with 1 intramuscular dinoprost (7.5 mg). Comparative analyses were conducted within individual antibiotic treatment groups, between each antibiotic and its respective control, and grouped comparing control versus antibiotic. Data were analyzed using generalized linear mixed models (GLMM) with significance set at $p < 0.05$. All mares ovulated 24 hours after GnRH treatment. Dismount parameters did not vary between control and ceftiofur (12.1 ± 5 ml and 11.7 ± 4.4 ml gel free volume; 71.7 ± 3.71 and 78.4 ± 3.6 total motility; 61.1 ± 8.3 and 66.7 ± 5.8 progressive motility, respectively) groups ($p < 0.05$) or between ticarcillin and control (12.3 ± 4.8 and 11.2 ± 5.5 ml gel free; 73.7 ± 5.27% and 70.1 ± 4.8% total motility; 65.5 ± 6.8% and 62.6 ± 8.1% progressive motility, respectively) groups ($p > 0.05$). Uterine infusions of ceftiofur or ticarcillin reduced ($p < 0.05$) endometrial leukocyte counts compared to their respective controls. Mares receiving antibiotics had fewer ($p < 0.05$) uterine infections (40.5%) than the control (54.2%) 72 hours after mating. Intrauterine fluid accumulation varied with time ($p < 0.05$) but not with groups or interactions ($p > 0.05$). The group overall affected the pregnancy rate ($p < 0.05$; antibiotic 36 versus 54% control). There was an effect of the group for ceftiofur ($p < 0.05$; ceftiofur 25 versus 75% control) but no effect of the group for ticarcillin (ticarcillin 56.6 versus 43.3% control; $p > 0.05$) for pregnancy rates. In conclusion, there was no association between dismount samples and mare clinical parameters. Despite apparent satisfactory semen parameters and lower leukocyte counts postmating in antibiotic-treated cycles, fertility was low.

Keywords: Mare, dismount semen sample, antibiotics, endometrial leukocytes

The unique framework of the equine fetal gonad for the synthesis of estrogen precursors

Katarzyna Malin,[a] Margo Verstraete,[a,b] William Holl,[c] Jamie Norris,[a] Machteld van Heule,[a,b] Kirsten Scoggin,[d] Barry Ball,[d] Mariano Carossino,[c] Alan Conley,[a] Pouya Dini[a]

[a]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, USA
[b]Department of Morphology, Imaging, Orthopedics, Rehabilitation and Nutrition, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
[c]Department of Pathobiological Sciences and Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
[d]Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA

At the eleventh month of equine pregnancy, fetal gonads deliver androgen precursors for estrogen synthesis to the placenta. Two of these estrogens, estrone and equilin, differ by 1 bond. This structural variation arises from their distinct synthesis pathways: estrone is derived from cholesterol, whereas equilin is derived from its precursor, 7-dehydrocholesterol (7-DHC). Because estrone and equilin synthesis periods overlap but peak at various stages of pregnancy, we hypothesized that the synthesis of their androgen precursors is separated spatially and temporally in the equine fetal gonad. To investigate how the estrogen profile in pregnancy is regulated, we conducted RNA-seq on 21 fetal gonad samples collected between the 4th and 11th months of pregnancy. We examined key elements in cholesterol synthesis (ACAT2, HMGCS1, HMGCR, MVK, PMVK, MVD, FDPS, FDFT1, SQLE, LSS, DHCR24, CYP51A1, LBR, TM7SF2, MSMO1, NSDHL, HSD17B7, EBP, SC5D, DHCR7) and early steroid hormone production (CYP11A1, CYP17A1). DHCR7—the enzyme that converts 7-DHC to cholesterol—was progressively downregulated throughout the pregnancy (Log2 fold change (FC) = −3.7; $p_{adj} < 0.001$), correlating with decreased estrone synthesis. Additionally, we noted less pronounced downregulation of the HMG-CoA reductase (HMGCR), and 24-dehydrocholesterol reductase (DHCR24), both involved in cholesterol synthesis, in the 10th versus 6th months of pregnancy (Log2FC=-2.3 and Log2FC=2, respectively, $p_{adj} < 0.05$). This suggested their role in the overall decline in steroid hormone synthesis observed towards parturition. No other differential expression was observed. We next asked if the synthesis of estrone and equilin precursors was separated spatially in the hormonally active gonadal interstitium. To test this, we first studied the interstitium for CYP11A1 and CYP17A1 expression via immunofluorescence to identify regions responsible for the synthesis of the androgen precursors. Next, we used RNA scope and immunofluorescence to investigate the potential compartmentalization of DHCR7 expression within this population. RNA scope had the highest mRNA compartmentalization in the 4th of pregnancy, with a decline with the progression of pregnancy, and immunofluorescence had less

Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723

pronounced compartmentalization in the male samples in the 6th month of pregnancy. These findings agree with our hypothesis and suggest that the expression of DHCR7 may be a limiting factor of the synthesis of estrone, and that equilin and estrone precursors are synthesized in separate compartments of the equine fetal gonad interstitium.

Keywords: Mare, fetal gonad, placenta, estrone, equilin, dehydrocholesterol

Delineation of miRNAs as biomarkers in equine chronic endometritis during different phases of the estrous cycle

Eden Manuel,[a] Alex Wittorff,[a] Lexie Russell,[a,b] Reed Holyoak[a]

[a]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA
[b]ViaGen Equine, Cedar Park, TX, USA

Chronic equine endometritis is a leading cause of subfertility in mares resulting in reduced foal production and financial losses. Endometritis is a dysbiosis either as a physiologic response to breeding associated contamination or another insult to the normal microbiome. However, due to immunological or mechanical abnormalities a chronic dysbiosis can develop, hindering the mare's reproductive success. Recent advances suggest associated roles of microRNAs (miRNAs) in the pathophysiology of chronic endometritis. Aim of this study was to further define the systemic expression profiles of these transcripts in normal (n = 5) versus chronically infected (n = 5) mares in the estrus versus diestrus stages of the estrous cycle. Mare cycle status was assessed regularly via transrectal ultrasonography. When the dominant follicle size reached 33-35 mm with appropriate signs of estrus, ovulation was induced using a GnRH analog, and whole blood samples were collected. Diestrous blood samples were collected 7 days after ovulation. Whole blood was collected into EDTA tubes and centrifuged (1,643 x g for 10 minutes). Plasma was separated into 1 ml aliquots and snap frozen in liquid nitrogen until processing. RNA was isolated from horse serum using the miRNeasy Serum/Plasma kit (Qiagen), RNA libraries were prepared using the TruSeq Small RNA Library Preparation Kit (Illumina), and sequencing was performed using the Illumina NextSeq 2000 platform. FASTQ files generated were mapped to horse mature miRNAs from the miRbase database using Bowtie software. Bioinformatic analysis is anticipated to demonstrate that mares suffering from chronic endometritis have dysregulated circulating transcript profiles that shift their physiological state towards a chronic inflammatory response that is pernicious to the events required to establish a successful pregnancy. Delineation of transcript nuances in mares experiencing chronic endometritis provides a baseline to further explore the development of useful biomarkers in judging the efficacy of therapeutics targeting chronic endometritis.

Keywords: Endometritis, horse, microRNA

Habitual physical activity of lean and overweight dogs throughout pregnancy measured with a triaxial accelerometer

Samantha McCarter,[a] Julie Cecere,[b] Orsolya Balogh[b]

[a]Department of Biomedical and Veterinary Sciences,
[b]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

Active lifestyle and moderate exercise have been broadly recommended by doctors for decades to women to promote general wellbeing and a healthier pregnancy and labor. Women who regularly engage in moderate intensity physical activity during pregnancy, including overweight and obese mothers, are able to mitigate the risks of several pregnancy-associated diseases such as gestational diabetes or the risk of a cesarian surgery.[1-3] Impacts of physical activity on the reproductive success and health of the dog and her pups are unknown, and consequently, there are no recommendations on what levels of physical activity are normal during pregnancy in the dog. We hypothesized that habitual physical activity gradually decreases throughout pregnancy in dogs. We also hypothesized that dogs with an overweight body condition begins pregnancy with a lower level of physical activity. Aims of this study were to determine habitual physical activity changes during pregnancy using a triaxial accelerometer, and to compare it between lean and overweight dogs. Medium to large breed (2-5 years), client-owned breeding dogs were enrolled and classified into lean (LE, body condition score (BCS): 4-5/9, n = 7) and overweight (OW, BCS: 6-7/9, n = 3) groups. All dogs were fitted with a FitBark 2 triaxial activity tracking device (FitBark Inc.) to their personal collar from shortly before ovulation to the end of parturition during owner's care. Accelerometer activity data was recorded as total cumulative daily activity quantified as 'BarkPoints'. BarkPoints were analyzed over the 9 weeks after ovulation (weekly averages) and during the last 7 days prepartum (daily totals) using mixed models; significance was set at $p < 0.05$. The baseline activity level of the dog was a significant determinant of their activity level throughout pregnancy. Average weekly BarkPoints were not significantly different over time. OW dogs had lower average weekly BarkPoints than the LE group. In the 7 days prepartum, daily total BarkPoints were significantly higher only in a day prepartum compared to 5 days prepartum, and without group effect. Although a definite conclusion cannot yet be drawn due to the small sample size, it appeared that pregnancy did not interfere with habitual physical activity level of dogs, and that an overweight status was associated with a more sedentary lifestyle. Owner lifestyle and perception of activity likely also affected these parameters.

Keywords: Dog, physical activity, pregnancy, accelerometer, body condition

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Comparing neonatal pups' growth between overweight and lean dogs

Samantha McCarter,[a] Stephen Werre,[b] Julie Cecere,[c] Orsolya Balogh[c]

[a]Department of Biomedical and Veterinary Sciences, [b]Department of Population Health Sciences, [c]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

Lactation is the final pathway for maternal metabolism to influence the neonate. In women, metabolic hormones positively correlate with maternal body mass index and these hormones pass through breastmilk. As a result, they affected infant weight gain through the first 6 months of life.[1] Although studies have examined the effect of many variables (i.e. breed, sex, etc.) on neonatal pup growth rates,[2] body condition of the dam has never been examined. We hypothesized that pups born and nursed by overweight dogs have increased growth rates compared to those from lean dogs. The aim was to compare growth rates of pups of lean and overweight dogs during the neonatal period. Thirteen litters from 12 healthy medium to large breed client-owned dogs were enrolled after whelping. Dogs were classified based on initial postpartum body condition into lean (LE, body condition score (BCS): 4-5/9, n = 6) and overweight (OW, BCS: 6-7/9, n = 7) groups. A total of 96 pups were born to LE (n = 48) and OW (n = 48) dogs. Birth weights and daily weights of pups in the litter were recorded by the owners until 21 days of age before weaning began. Pups were solely nursed and did not receive supplemental feeding. Birth weight, daily body weights, average daily weight gain (ADG, %), and average weight gain since birth (AGB, %) of pups were analyzed using mixed model ANOVA; significance was set at $p < 0.05$. Pup birth weights were lower in larger litters but unaffected by maternal BCS. Pup growth curves based on daily weight changes were significantly different between the 2 maternal groups, despite pup weights not being different on any given day between OW and LE mothers. ADG on days 2 and 4 was 6% and 3.1% higher, respectively, in pups of OW dams compared to LE, whereas pups of LE dams gained 2.2% more on day 13. AGB did not differ significantly on any given day between BCS groups; however, tripling and quadrupling of birth weights occurred ~ a day later in pups of OW dogs. A larger litter size accounted for significantly lower AGB. Pups with higher birth weights had generally higher weights throughout the neonatal period, although they were gaining (ADG, AGB) at a slightly slower rate. In conclusion, despite the similar neonatal body weights between maternal groups, pups from OW dams grew differently in the first 21 days compared to pups from LE dogs. Litter size or birth weight are also significant determinants of pup weight and growth. Milk composition may explain some of the differences observed in growth pattern between pups from OW and LE dogs and is currently investigated.

Keywords: Dog, growth rate, average daily gain, pup, neonate, body condition

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Impact of canine obesity on maternal insulin resistance and fetal metabolic profile

Autumn Mendoza, Linda Nduwimana, Jenny Lin, Bryce Gilbert, Viviane Gomes

Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA

Obesity affects nearly 60% of domestic dogs in the US. In humans, obesity is associated with increased pregnancy insulin resistance, leading to fetal hyperinsulinemia, disrupted growth, neural, cardiac, and pancreatic development. The impact of obesity in pregnant dogs and their offspring is poorly understood. We aimed to elucidate glucose and insulin profiles in dogs and offspring affected by obesity. We hypothesized that obese (Ob) dogs and their fetuses exhibit hyperglycemia and hyperinsulinemia compared to lean (Le) controls. Female Beagles assigned to Ob and Le groups were fed ad libitum or to meet energy requirements, respectively (n = 3-5/group). Obesity was defined as ≥ 20% weight gain. Fasting blood glucose was measured during anestrus (A), proestrus/early estrus (P/E), early, mid, and late-pregnancy using a point-of-care glucometer (Precision Xtra, Abbott). Intravenous glucose tolerance tests (IVGTT) were performed during P/E and on day 46 of pregnancy (LH surge = d0). Glucose and insulin were measured at baseline (-5 minutes), and 1, 5, 10, 20, 30, 60, and 90 minutes after intravenous dextrose treatment (0.5 g/kg bodyweight). On days 56-63, dogs underwent cesarian surgery and fetal glucose/insulin were measured (4 fetuses/litter, n = 12-16/group). Insulin was measured via radioimmunoassay (HI- 14K, Millipore Corporation). ANOVAs and Tukey's tests were performed (GraphPad Prism 10.1.2). In Le and Ob dogs, fasting glucose was higher at mid and late-pregnancy than at A, P/E, and early-pregnancy ($p < 0.05$). Both Le and Ob dogs presented greater IVGTT glucose area under curve (AUC) during pregnancy than P/E. Only Ob dogs presented greater ($p < 0.05$) insulin AUC in pregnancy versus proestrus that was also higher than Le pregnancies. Glucose and insulin concentrations were not different ($p > 0.05$) between Le and Ob fetuses. In conclusion, obesity appeared to enhance insulin resistance in pregnant dogs, with unaffected late-pregnancy fetal insulin and glucose concentrations. Further research is warranted to explore maternal obesity impact in canine offspring.

Keywords: Metabolism, pregnancy, adiposity, insulin, maternal, fetal

Identification and management of an atypical granulosa cell tumor in a pregnant mare

Brittany Middlebrooks, Peyton Draheim, Richard Holder, Kristina Lu

Hagyard Equine Medical Institute, Lexington, KY, USA

Granulosa cell tumors (GCT) are typically diagnosed when transrectal palpation and ultrasonography reveals a unilaterally enlarged ovary with the contralateral ovary being inactive and small.[1] Diagnosis of a GCT is based on history, transrectal palpation, ultrasonography, and hormone evaluation; however, diagnosis of early or atypical GCTs can be challenging.[2] A 15-year, Thoroughbred mare, foaled in early 2023 and was later treated for bacterial endometritis. Following treatment, the mare was bred, but no pregnancy achieved. Three weeks later, transrectal palpation and ultrasonography revealed a firm right ovary with a cystic structure as the only notable abnormality. Serum was submitted for antimüllerian hormone (AMH), inhibin B, and testosterone concentrations. Results were within normal limits for a nonpregnant, cycling mare; although, inhibin B was approaching the upper limit of the reference range. A subsequent evaluation 2.5 months later revealed that the cystic structure on the right ovary has regressed completely. Both ovulation fossae were palpable, and the ovaries were small and firm, with a corpus luteum on each ovary. GCT panel was repeated that was within normal limits. In 2024, the mare was bred, ovulated from both ovaries, and was diagnosed with twins 13 days later. One of the vesicles was manually reduced and a single remaining embryonic vesicle was identified. Pregnancy was monitored via transrectal ultrasonography until day 60 of pregnancy when the mare had a markedly enlarged left ovary. Third GCT panel was submitted and testosterone and AMH concentrations were both elevated, suggestive of a GCT. The elevation in testosterone was consistent with a theca cell component; however, laboratory reference ranges are provided only for nonpregnant mares. Pregnancy monitoring occurred monthly throughout pregnancy and the ovaries were assessed via transrectal and transabdominal ultrasonography. Ovary fluctuated in size and ranged from 6.9-15 cm. Another GCT panel was submitted at ~ 190 days pregnancy and only testosterone concentrations were elevated. Mare foaled in 2025. Six days after foaling, the left ovary was firm and enlarged with no palpable ovulation fossa. Fifth GCT panel was submitted, and testosterone concentrations were elevated, supporting the diagnosis of a granulosa theca cell tumor (GTCT). Transrectal ultrasonography 13 days after foaling revealed ovulation on the left ovary with an appreciable tumor-like structure and a small, inactive right ovary. Treatment (ovariectomy) of a GTCT is performed by colpotomy (flank, ventral midline, or paramedian surgical approaches) or by laparoscopic approaches.[1] In this case, surgery was considered when the ovary was first noted to be enlarged, but there were concerns regarding pregnancy loss. Ovarian size was monitored throughout pregnancy to determine if the ovary was approaching a diameter that would have been too large to remove via flank approach. These efforts were made in hopes to avoid surgery via ventral midline incision due to the complications that can arise.[3] This case described an atypical presentation of a GTCT and highlighted the difficulty in diagnosing these cases, along with the need to consider the pros and cons of surgical and laparoscopic approaches for ovariectomy.

Keywords: Granulosa cell tumor, mare, surgery, pregnancy

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Investigation into hypochlorous acid as a treatment for bacterial endometritis in the mare

Kayleigh Moore,[a] Hannah Cohen,[a] Ryan Oliveira,[b] Jenny Sones,[c] Brendon Meldrum,[d] Trevor Alexander,[d] Claire Burbick,[b] Babiche Heil[a]

[a]Veterinary Clinical Sciences, [b]Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA, USA
[c]Clinical Sciences, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA
[d]Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA, USA

An increase of antimicrobial resistance necessitates alternative treatments to bacterial infections. Investigated in this pilot study; hypochlorous acid (HOCl), an endogenous substance with broad- spectrum antimicrobial activity to treat bacterial endometritis. We hypothesized that HOCl is effective at killing bacteria known to cause equine endometritis and can be used intrauterine at 240 ppm without detrimental effects in the mare. Aims of this study were to: 1. Investigate if HOCl (Wound and Skin Care Liquid, Vetericyn Plus VF. Innovacyn, Inc.) is effective at killing bacteria known to cause equine endometritis; and 2. can 120 ppm and 240 ppm HOCl be safely used for intrauterine irrigation in the mare. Broth microdilution assays were used to establish the minimal inhibitory concentration (MIC) for gram-positive *Streptococcus equi* subsp. *zooepidemicus* and *Enterococcus faecalis*, and gram-negative *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Six estrous mares received once daily intrauterine infusions of 120 ppm and 240 ppm HOCl (n = 3/group) for 3 days. Endometrial cytology score (neutrophils/100 endometrial cells) and vaginoscopy were performed before, during, 1 and 23 days after treatment. Serum amyloid A (SAA), fibrinogen (Fib) and white blood cell (WBC) were performed before, during, and 1 day after treatment. Endometrial biopsy was performed before, 1 and 23 days after final treatment and assigned Kenney-Doig score. We established a MIC for gram-positives at 200 ppm and gram-negatives at 225 ppm. Further results reported in (median, IQR). No side effects were noted, and no differences between 120 ppm and 240 ppm groups for SAA (< 20 mg/l, 0 mg/l), Fib (266.7 mg/dl, 50 mg/dl; 200 mg/dl, 1.5 mg/dl), WBC (6.4 x 10^3/µl, 0.46 x 10^3/µl; 7.7 x 10^3/µl, 1.18 x 10^3/µl). Five out of 6 mares had a decline in cytology score during treatment whereas 1 out of 6 mares had a gradual increase. Four out of 6 mares had no change in Kenney-Doig score over the study period. One out of 6 mares

had an ulcerative lesion that resolved, and 1 out of 6 mares changed from grade 1 to 2B over the study period. In summary, HOCl was effective in killing common bacteria causing equine endometritis and it could be a safe, nonantibiotic treatment in mares.

Keywords: Horse, endometritis, treatment, hypochlorous acid

Hock umbilical cord entanglement in an Angus calf

Kayleigh Moore, Ilaria Colombo, Ahmed Mosallam, Salman Waqas, Michela Ciccarelli

College of Veterinary Medicine, Washington State University, Pullman, WA, USA

A 6-year, multiparous Black Angus cow was presented for dystocia. Vaginal examination revealed a deceased fetus in anterior longitudinal presentation, dorsosacral position with bilateral shoulder flexion. Fetid fluid was detected in the vaginal vault. A caudal epidural anesthetic was given, and the fetal malposition was promptly corrected. However, extraction of the calf remained unsuccessful. Upon further evaluation, one of the fetus's hind limbs was entrapped within the birth canal due to the umbilical cord being tightly wrapped around the hock. A blind resection of the umbilical cord was performed, allowing the limb to be freed and the fetus delivered. The heifer calf was fully developed but showed distal limb swelling beyond the site of entrapment, along with a distinct indentation where the umbilical cord had constricted the limb. Additionally, an omphalocele was observed. Postpartum vaginal examination revealed the presence of a second fetus, which was delivered successfully and found to be a healthy heifer. Umbilical cord abnormalities are well-documented causes of abortion and stillbirth in equines. An excessive umbilical cord length (> 85 cm) can predispose to umbilical cord torsion in horses. However, reports of cord entanglement around fetal limbs in equine pregnancy are rare, with only 1 documented case.[1] In human obstetrics, umbilical cord entanglement is relatively common, often occurring around the neck (nuchal cord), and is not associated with the length of the cord.[2] A case of fetal demise due to the nuchal cord was reported in a camel, leading to abortion.[3] In cattle, the umbilical cord is relatively shorter than in equines, with an average length of approximately 28 cm, making such abnormalities very rare. To our knowledge, this is the first reported case of umbilical cord entrapment leading to dystocia in cattle. This condition should be considered a potential fetal factor contributing to malposition during dystocia management.

Keywords: Cattle, obstetrics, umbilical cord abnormalities, dystocia,

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Ovarian remnant syndrome in a cat with inconclusive diagnostics

Hayley Moore,[a] Diane Delmain,[a] Lily Lewis,[a] Alan Conley,[b] Robyn Wilborn[a]

[a]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA
[b]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, USA

A 2-year, spayed female domestic shorthair cat, was presented for evaluation of estrous behavior, including lordosis, vocalization, and rolling, that occurred on a cyclical basis every 3 weeks. Clinical signs began ~ 6 months after ovariohysterectomy (OHE) performed at 19 months of age. An exploratory abdominal surgery by the referring veterinarian at 11 months after original OHE revealed no ovarian tissue. On evaluation at referral hospital, serum was submitted for antimüllerian hormone (AMH) and the concentrations were 0.14 ng/ml, consistent with a spayed female (reference range in ovariectomized cats: 0.01-0.16 ng/ml). Progesterone concentrations and were 1.3 ng/ml, consistent with no luteal tissue. Cat returned 3 weeks later for follow up examination while she was exhibiting estrous behavior. Vaginal cytology had 90% cornification of the vaginal epithelial cells. Serum was submitted for AMH and results were once again consistent with a spayed female (0.10 ng/ml). Cat was given intramuscular gonadorelin (Fertagyl, Merck Animal Health) (43 µg once) to induce ovulation. Cat returned for serum progesterone 3 weeks after gonadorelin treatment. Serum progesterone concentrations were 1.6 ng/ml, consistent with no luteal tissue. Despite the inconclusive diagnostics, an abdominal exploratory was performed based on clinical signs and confirmation of estrogen via vaginal cytology. A small, 3 mm piece of ovarian tissue was confirmed on histopathology at the location of the right ovarian pedicle. The tissue contained follicles in all stages of development and a single mature corpus luteum. This case study demonstrated that a very small amount of ovarian tissue can produce adequate estradiol to stimulate estrous behavior in the cat and produce cornification of vaginal epithelial tissue yet produce serum AMH of that seen in ovariectomized cats and a single corpus luteum can produce serum progesterone consistent with no luteal tissue generally observed in an intact cat.

Keywords: Ovarian remnant, cat, antimüllerian hormone, progesterone

A detailed characterization of *Streptococcus zooepidemicus* mechanism of infection during equine placentitis

Hugo Monteiro,[a] Machteld van Heule,[a,b] Rodrigo Profeta,[a] Jamie Norris,[a] Anneliek ter Horst,[a] Kinga Graniczkowska,[a] Mariano Carossino,[c] Kirsten Scoggin,[d] Claire Shaw,[a] Fabio Lima,[a] Richard Pereira,[a] Barry Ball,[d] Titus Brown,[a] Peter Daels,[b] Bart Weimer,[a] Pouya Dini[a]

[a]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, USA
[b]Department of Morphology, Imaging, Orthopedics, Rehabilitation and Nutrition, Faculty of Veterinary Medicine, University of Ghent, Merelbeke, Belgium
[c]Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
[d]Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA

Several methods have been employed to study pathogen infections. Here, we demonstrate how the integration of multi-omics alongside strict experimental settings allows for a detailed characterization of host-pathogen interactions. Specifically, we leveraged ultradeep sequencing in a prospective randomized controlled trial to evaluate the interaction of *Streptococcus equi* subs. *zooepidemicus*, as a primary pathogen of equine placentitis (chorioallantois) with the host. We hypothesized that *S. zooepidemicus* establishes infection through crossfeeding with other microbes that reside in the placenta. Six out of 12 healthy pregnant mares at 272 days of pregnancy were randomly assigned to receive an inoculation of a *S. zooepidemicus* isolated from equine placentitis, whereas the remaining 6 mares served as negative controls. The progress of the disease was monitored for 8 days before placental samples were collected for ultradeep shotgun-DNA and dual-RNA sequencing, primary metabolomics, and in situ hybridization (ISH) analyses. Samples were sequenced at 200 million reads depth for shotgun-DNA and 150 million reads for dual-RNA sequencing. The inoculated isolate was also sequenced for whole-genome versus metagenome- assembled genome (MAG) comparisons before and during the in vivo establishment of the disease, respectively. The probes used for ISH were designed based on the assembled 16S gene from the genome, MAG, overall Streptococcus sp., and recovered bacteria. Microbial contaminants were assessed by sequencing reagent samples and a probe in ISH. A bioinformatics pipeline was calibrated for 100% accuracy in microbial species identification, using a published mock community and the genome of *S. zooepidemicus*. In this pipeline, host DNA and RNA reads were carefully separated from microbial reads using various bioinformatic approaches and the horse reference genome from the National Center for Biotechnology Information (NCBI). Genes were de novo assembled using microbial RNA reads and aligned against the nucleotide database from NCBI to further remove potential eukaryotic genes. Microbial classification in a highly strict mode using DNA and RNA reads revealed that another *Streptococcus* sp. potentially resided in the placenta, such as *S. vestibularis* and *S. dysgalactiae*, or a potential new species of uncultured *Streptococcus*. A total of 1,213 microbial genes were recovered, of which 233 were differentially expressed in mares with placentitis. During infection, several microbial species had upregulated genes related to nutrient acquisition, metabolic adaptation, stress response and survival, growth, and virulence factors, but *Streptococcus* genes accounted for most upregulated ones (n = 186) from which also had adhesion and colonization genes upregulated. These genes, alongside the identified metabolites, point to microbial degradation of the placenta extracellular matrix (ECM) as the mechanism of infection, in which ECM regeneration was confirmed to be one of the main pathways upregulated in placentas with placentitis. Further analyses suggested that *S. zooepidemicus* undergo single-nucleotide (SNP) modifications, including their 16S gene. In situ hybridization analyses confirmed the presence of *Streptococcus* species in the placenta and further confirmed SNP modifications. Overall, these analyses revealed that *S. zooepidemicus* may not be the only Streptococcus in the placenta during equine placentitis and that other microbes may reside in healthy placentas.

Keywords: Metatranscriptomics, metagenomics, ultradeep sequencing

Polycystic/fibrocystic mastopathy following ovariohysterectomy in a diestrous dog

Kara Mosier,[a] Ann Marie Rizzo,[a] Joshua Borg,[a] Gail McRae,[b] Viviane Gomes[a]

[a]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA
[b]Leader Dogs for the Blind, Rochester Hills, MI, USA

A 3.5-year, female Pug underwent ovariohysterectomy during diestrus, ~ 4-5 weeks after last estrous cycle. One month after surgery, the dog developed acute mammary gland enlargement, characterized by multiple cystic structures distributed across both mammary chains and bloody mammary discharge. Bloodwork abnormalities included reticulocytosis and mild leukocytosis with neutrophilia and monocytosis. Red blood cells, degenerative neutrophils, lymphocytes, and macrophages with a granular background were noted on mammary discharge cytology. The dog was given a 10 day course of oral amoxicillin-clavulanate (12.5 mg/Kg; once every 12 hours) but failed to improve. Follow-up mammary discharge cytology revealed neutrophils, lymphocytes, foam cells/macrophages, and few extracellular bacteria. Culture yielded mild growth of *Staphylococcus pseudointermedius*, susceptible to amoxicillin-clavulanate. Dog was then prescribed a 7 day course of oral amoxicillin-clavulanate (12.5 mg/Kg; once every 12 hours), 5 day course of oral carprofen (2.2 mg/Kg; once every 12 hours), and cold compresses, with minimal response. On ultrasonography examination, multiple cystic cavities ranging 0.32-3 cm in diameter were diffusely distributed throughout both mammary chains. The cystic cavities contained hypoechoic to mildly echogenic fluid, with larger cysts also containing hyperechoic sediment. There was complete absence of normal mammary architecture. Ultrasound-guided aspirations were performed, yielding 12 ml of serosanguinous discharge and yellow caseous material. Fluid sediment contained numerous poorly preserved neutrophils, foam cells/macrophages within a pink proteinaceous material, and lysed cells. Few macrophages contained hematoidin crystals. No etiologic agents or atypical cell populations were observed. Findings were consistent with polycystic or fibrocystic mastopathy, a typically benign mammary dysplasia rarely observed in dogs. This condition has been associated with hormonal fluctuations, particularly estrogen-progesterone shifts, or progestin supplementation. However, in this case, there was no history of exogenous steroid hormone exposure

and hormone concentrations (progesterone: 0.29 ng/ml, LH: > 1 ng/ml) did not support ovarian remnant syndrome. This case presented a firsthand report of polycystic/fibrocystic mastopathy as a complication of ovariohysterectomy performed during diestrus.

Keywords: Dog, mammary gland, blue dome, cysts

Granulosa cell tumor in a yearling Angus donor heifer

Cameron Nau, Alexandra Grillos, Erin Runcan

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

Granulosa cell tumors (GCT) are one of the most common ovarian tumors in cattle with a prevalence rate of 0.5%.[1] The appearance and size of these tumors are variable with some masses measuring > 30 centimeters in diameter.[1,2] Malignancy is rare.[3] Clinical signs of GCT cases can vary, but may include anestrus, masculinization, udder development, lactation, and nymphomania.[4] A 1-year, Aberdeen Angus heifer, was presented for evaluation of an enlarged left ovary. One week before, the heifer had presented for follicle aspiration at a commercial embryo transfer facility. It was discovered at that time that the left ovary was enlarged and unable to be aspirated. On presentation, vitals were within normal limits, and presented with a history of persistent signs of estrus. Transrectal ultrasonography of the reproductive tract revealed an enlarged, abnormal left ovary measuring 9 cm with a small, inactive right ovary. Top differentials for the abnormal ovary included granulosa cell tumor, fibroma, and teratoma. Surgical removal of the affected ovary was elected and performed via standing laparoscopy. Ovary was submitted for further diagnostic evaluation that confirmed the suspected diagnosis of GCT. Three months after ovariectomy, the heifer was introduced to a bull and became pregnant; calved during the winter of 2024 with no complications.

Keywords: Heifer, granulosa cell tumor, ovariectomy

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Estradiol cypionate-sulpiride treatment to seasonally noncycling mares: the endocrine response

Erin Oberhaus,[a] Isabella Faulkner,[b] Jennifer Hatzel,[b] Sarah Ward,[b] Patricio Razquin,[b] Javier Funes,[b] Brody Klinglesmith,[b] Pat McCue,[b] Jenny Sones[b]

[a]School of Animal Sciences, Louisiana State University, Baton Rouge, LA, USA
[b]Equine Reproduction Laboratory, Department of Colorado State University, Fort Collins, CO, USA

Advancing the first ovulation of the year in seasonally anovulatory mares has substantial impact on equine breeding programs. Artificial light is a reliable method to hasten transition into the breeding season but can be challenging for large groups of mares maintained outside. Combination of estradiol and the dopamine antagonist, sulpiride, in a long-acting vehicle stimulated prolactin and luteinizing hormone (LH) in deep anestrous mares, resulting in rapid follicle growth and advancement of first ovulation of the season. Original studies evaluating the ECP-sulpiride combination were conducted in the southern United States. Effects of different climates, latitudes, and elevations have not been assessed. We hypothesized that estradiol cypionate (ECP) and sulpiride stimulates prolactin and LH in deep anestrous mares in a semi-arid, continental region (i.e. Northern Colorado) similar to mares in the South. Beginning in January, 12 mares (10-19 years) housed in northern hemisphere paddocks without artificial lighting were enrolled in a crossover study. Mares were randomized to treatment (ECP (50 mg) on day -1 and sulpiride (3 g) on day 0, both intramuscularly) and vehicle (n = 6/group). Jugular blood sampling began on day of ECP treatment and continued for 11 successive days. Two weeks later, previous control mares received an identical treatment of ECP-sulpiride, and blood sampling repeated. Plasma prolactin and LH were determined via radioimmunoassay. Two-way ANOVA was used to compare prolactin and LH between treated and control mares, and between mares receiving ECP-sulpiride 2 weeks apart. A treatment by day interaction was observed for both prolactin ($p < 0.0001$) and LH ($p < 0.0001$). Prolactin was stimulated beginning 1 day after sulpiride treatment and remained elevated for 9 days. Plasma LH was stimulated beginning 5 days after sulpiride treatment and remained elevated until blood sampling concluded on day 10. Prolactin and LH responses were similar in mares treated 2 weeks apart. In this study, treatment of noncycling mares with ECP-sulpiride stimulated a robust rise in prolactin and LH that persisted for at least 9 days. The magnitude and duration of prolactin and LH secretion were similar to previous observations in a subtropical region, providing evidence that harsher winter climates and higher altitudes may not hinder the stimulatory effects of ECP-sulpiride.

Keywords: Mare, season, sulpiride, prolactin

Caruncular edema and torsion in an ewe

Rachael O'Connell,[a] Rachael Gately,[a] Adam Ward,[a] Robert Dwyer,[a] Christopher Premanandan,[b] Carlos Pinto[a]

[a]Department of Ambulatory Medicine and Theriogenology, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA
[b]Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

A 7-year, Icelandic ewe, was presented for a 30 day history of frequent straining and hind end bloody discharge. Historically, the ewe successfully bred naturally and lambed uneventfully 3 times. On presentation, the findings of physical examination were within normal limits with the exception of frequent unproductive straining, serosanguinous vaginal discharge, and asymmetrically enlarged udder. Vaginal speculum examination revealed dark red to black serosanguinous discharge from the cervix. Transabdominal ultrasonography revealed enlarged uterus with intraluminal hypoechoic fluid. Ovariohysterectomy was performed under general anesthesia. On gross examination, the uterus was moderately and uniformly distended with an obvious firm mass within the left uterine horn. Incision of the uterus revealed serosanguinous fluid and prominent pinkish caruncles (average 12 mm diameter) throughout with one 25 mm diameter darkened pedunculated mass corresponding to a caruncle. No obvious ovarian abnormalities were noted. Uterus and ovaries were submitted for histopathology examination. Ovaries had follicular development with primordial, primary and secondary follicles along with a corpus albicans in both ovaries. There was marked widespread caruncular edema. The dark mass was a caruncle distended with hemorrhage indicative of coagulation necrosis resulting from torsion of the caruncle's base. There were no other predisposing causes of vascular impairment that would lead to the severe caruncular edema, hemorrhage, and necrosis. According to a recent literature search, torsion of caruncles has not been previously reported, and its etiology remains to be elucidated. The ewe was discharged the same day, and her clinical signs completely resolved immediately following surgery according to the client during a follow up phone call 2 months later.

Keywords: Sheep, caruncle, edema, torsion, ovariohysterectomy

Use of endotracheal tube as a long-term stent for chronic pyometra in a mare

Kornelia Omyla, Julianne Nussbaum, Amanda Ayers, Carrie Palm, Dennis Woerde, Catherine Renaudin, Pouya Dini

School of Veterinary Medicine, University of California, Davis, CA, USA

Pyometra in mares is a chronic inflammatory condition characterized by the accumulation of inflammatory fluid in the uterus due to impaired uterine clearance, cervical dysfunction, and/or poor vaginal conformation. Unlike in other species, pyometra is relatively rare in mares but poses a substantial risk for subfertility or infertility. The prognosis for restoring fertility is poor, especially in cases of cervical closure leading to a closed pyometra with extensive intrauterine fluid accumulation. Treatment options include medical management and surgical intervention, with ovariohysterectomy as the most definitive but challenging procedure due to the risk of postoperative complications. A 17-year, Warmblood maiden mare, was presented with chronic pyometra, with the main complaint being discomfort during riding with frequent urination. On examination, cervical adhesion and abnormalities were diagnosed using digital manipulation and endoscopy. Uterine lavages and cervical dilations had been previously performed multiple times without success. As breeding was not intended for the mare, an endometrial biopsy was not performed. Due to the mare's age, and the cost and risks of an ovariohysterectomy, the owner opted for a nonsurgical option. A sterile 9.5 mm cuffed endotracheal tube was placed inside the cervix after manual dilation, and the cuff was inflated with sterile water. Accurate placement was confirmed via transrectal ultrasonography and verified at 1 week, 3 weeks, 2 months, 6 months, and 1 year. Subsequent reexaminations for up to 1 year revealed no fluid within the uterus, effective uterine drainage and appropriate cervical tube position. This report highlighted a case in which an endotracheal tube was successfully used as a cervical stent following manual cervical dilation to maintain continuous uterine drainage. The mare remained symptom-free for up to 1 year, demonstrating good clinical progress and normal performance. Although potential complications, such as stent loss, exist, the cervical stent provides a simple, cost-effective, and minimally invasive alternative for long-term management of pyometra, particularly in cases where surgery and general anesthesia pose substantial risks or where financial constraints are present.

Keywords: Pyometra, mare, cervix, stent, nonsurgical treatment

Assessment of fetal ultrasonographic parameters for predicting parturition date in mares

Kornelia Omyla, Catherine Renaudin, Pouya Dini

School of Veterinary Medicine, University of California, Davis, CA, USA

Foaling is a rapid event and any complications can quickly compromise the foal. This necessitates the presence of skilled personnel to assist or promptly detect dystocia, ultimately improving the survival rates of the offspring. The variable length of pregnancy in mares makes predicting the foaling date challenging. The pH and calcium concentrations of mammary secretions are commonly used but still lack reliability in some cases. We aimed to utilize certain fetal developmental characteristics to enhance the accuracy of foaling date prediction. Healthy pregnant mares (n = 8) with known ovulation dates were monitored weekly from day 310 of pregnancy to parturition by transabdominal and transrectal examinations. Transrectal ultrasonography was performed to evaluate the size of the navicular bone, as well as the diameters of the internal (IOS) and external (EOS) cervical os. Transabdominal ultrasonography was performed to assess the timing of fetal stomach rugae appearance and sustained gastrointestinal peristalsis lasting over 30 seconds. On presentation of mammary secretions, calcium concentrations and pH were measured using a Foal Watch test kit and a commercial

pH meter. Regression analysis was performed to evaluate the relationship between the measured parameters and the number of days to parturition. Navicular bone size was significantly associated with the number of days to parturition (R^2 = 0.52, $p < 0.001$). Both pH (R^2 = 0.36, p = 0.003) and calcium concentrations (R^2 = 0.35, p = 0.007) had moderate association with days to parturition. The diameter of IOS, EOS and GI peristalsis scores were also significantly associated with days to parturition (p = 0.02, p = 0.04, and p = 0.005, respectively). However, their predictive power was weak (R^2 = 0.1, R^2 = 0.1, and R^2 = 0.2, respectively). The presence of stomach rugae was scored on a scale from 0 to 2 (0- stomach rugae not present; 1-stomach wall slightly irregular; 2- stomach rugae present). A significant association was there between the rugae scores and days to parturition (R^2 = 0.35, $p < 0.0004$), with mares displaying a score of 2 predicted to foal within 6 days. Results demonstrated that among the examined parameters, navicular bone size was the most reliable predictor of parturition timing, whereas pH, calcium concentrations, and gastrointestinal scores had moderate associations. Further validation is needed to enhance predictive accuracy in equine reproductive management.

Keywords: Pregnancy, foaling, parturition, mare, ultrasonography

Femoral dysgenesis in a neonatal Weimaraner pup

Acadia Parker,[a] Kristin Eden,[b] Orsolya Balogh,[a] Julie Cecere[a]

[a]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA
[b]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

A 4-day, Weimaraner male pup, weighing 207 grams, was presented for routine breed-associated surgery; it was considerably smaller than littermates since birth, but was vigorous and gaining weight. On physical examination swelling and pain on palpation of the right femur and stifle were noticed. Based on findings, no further diagnostics or surgery were performed, and he was discharged with injectable buprenorphine and oral amoxicillin-clavulanic acid. Three days later, pup returned due to lack of improvement. Radiographs revealed a misshapen, lytic distal right femoral epiphysis. Based on the constellation of clinical and radiographic findings, humane euthanasia was elected due to lack of response to treatment and poor long-term prognosis. Necropsy was performed, and gross evaluation revealed an eroded distal right femur suggestive of osteolysis. Histology revealed extensive effacement and replacement of the distal femur architecture by densely packed, mitotically active mesenchymal cells, all in various stages of maturity. Aerobic culture of the synovial fluid had no bacterial growth. Given the lack of inflammation or infection, and the persistence of an early mesenchymal tissue core that tightly interdigitated with existing tissue, the pup was diagnosed with congenital developmental dysgenesis of the distal femur. Conditions concerning appendicular skeletal dysgenesis in puppies are rare and scarcely documented.[1] Underlying causes of these defects in dogs have been poorly defined, but are hypothesized to be related to dam nutrition, fetal positioning, and failures of intrinsic embryogenesis and cellular differentiation.[2] Instances of canine axial skeletal malformation are well-documented, with the mechanisms of these defects more clearly delineated. The scarcity of appendicular skeletal malformations in literature suggests the need for greater investigation and testament of them. This case serves as a well-documented clinical, radiographical, and histological example of the lesser-investigated pelvic limb variant of canine appendicular skeletal defects.

Keywords: Dog, neonate, pup, femoral dysgenesis, appendicular skeleton

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Vulvar injection of 2.5% iPAAG Hydrogel to improve perineal conformation

Lauren Pasch

Rhinebeck Equine, Rhinebeck NY

A multiparous barren 10-year, Thoroughbred mare with evidence of vulvar and vestibular trauma. was presented for breeding management. There was a dehisced Caslick in place which failed to provide a barrier to the internal reproductive tract. The mare's uterus was completely distended with air to the tips of both uterine horns, preventing transrectal ultrasonographic imaging of the uterus. Multiple small follicles were present on each ovary and the mare was hyperreactive to any vulvar manipulation. Over a 1 year period, multiple Caslick operations were performed, reinforced, repaired, and ultimately destroyed when the mare aborted. At the beginning of the following breeding season, 10 milliliters of ArthramidVet 2.5% polyacrylamide hydrogel was injected into the dorsal vulvar lips at the level of the perineal body. A visible bulge was appreciated at the injection sites, and a temporary horizontal mattress suture was placed just dorsal to the perineal body to improve the vulvar seal. The mare was bred via live cover and double ovulation was confirmed. After ensuring uterine fluid clearance, a Caslick operation was performed with additional horizontal mattress sutures to relieve tension on the primary sutures. The mare was confirmed pregnant with twins at 14 days post ovulation and a manual twin reduction was performed; the Arthramid-induced bulges remained visually appreciable at this time (27 days post injection). The vulvar seal remained adequate, as evidenced by lack of intravaginal air seen on repeat ultrasound examinations. The injection site bulges were grossly visible for approximately 90 days. At 120 days following Arthramid injection, the Caslick and horizontal mattress sutures were removed, leaving fully sealed vulvar lips. The injection site bulges were no longer visually appreciable. A pinpoint defect was evident at the level of the perineal body; the edges were freshened and a single suture placed to address the defect. The mare was pregnant as of routine fall

Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723

examination. This case described the use of a hydrogel product to increase the tissue bulk of the vulvar lips. This created enough tissue bulk and vulvar seal to allow for live cover breeding, standard breeding management of the mare, and the Caslick to heal without being under tension. The downsides of this technique are the volume of hydrogel, in that a volume greater than 10 ml would be beneficial but is cost prohibitive for most clients, as well as the finite lifespan of the product, so in some cases re-application may be necessary after 90-100 days. Potential long term effects on the character of vulvar tissue have yet to be evaluated.

Keywords: Mare, Caslick, vulvoplasty, hydrogel

Seminal microbiome and its influence on the mare uterine microbiome

Giorgia Podico, Igor Canisso

Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA

The functional role of the semen microbiome has been explored in women but not in domestic species; studies demonstrated its interaction with the vaginal and uterine microbiome changes the microbiome composition and effects on physiological functions and fertility. High bacterial richness in semen was linked to dysbiosis in the vaginal microbiome of couples and to cases of subfertility. This study was designed to study the effects of the seminal microbiome on the mare uterine microbiome; we hypothesized that the semen and seminal plasma microbiome of the stallion differs from the jack and that could trigger distinct changes in the mare uterine microbiome. Fertile mares (n = 15) were serially monitored using transrectal ultrasonography to detect signs of estrus. Once in estrus, mares were inseminated once with fertile jack or stallion raw semen (2×10^9 total sperm) with seminal plasma from either male or saline in a crossover design. A sample of uterine fluid was collected before insemination, at 6 and 24 hours after insemination, and at embryo flush (8 days after ovulation). Bacterial DNA was purified from semen, seminal plasma, and uterine fluid with a commercial kit and submitted for PCR amplification and sequencing of the full-length 16S region. Taxonomy assignments were done via DADA2 using the Silva database, and R was used for the analyses. The dataset was filtered and agglomerated per rank to the family level. Alpha and beta diversity indexes were calculated and compared with the Wilcoxon rank-sum test, a linear mixed model, and PERMANOVA; significance was set at $p < 0.05$. Alpha diversity (Faith's PD index) differed ($p = 0.048$) between donkey and horse semen, whereas beta diversity was not different ($p > 0.05$) across species. The most prevalent phyla in mares bred to the donkey were *Proteobacteria* (50.3%), *Actinobacteria* (18%), and *Firmicutes* (29.8%). Similarly, in mares bred to the horse, *Proteobacteria* (62.5%), *Actinobacteria* (21.7%) and *Firmicutes* (12.3%) were the most abundant. Noteworthy, bacteria from the order of *Lactobacillales* contributed up to 20% of the microbiome composition in mares bred to the donkey and up to 6% in mares bred to the horse. Twenty-four families of bacteria were in common between donkey semen and the uterus after the insemination, and 9 were identified between horse semen and the uterus. Species richness and evenness (alpha diversity) were not different in mares with a positive or a negative embryo flush (Observed ASVs, $p = 0.11$; Chao1, $p = 0.22$; Shannon, $p = 0.91$; Simpson, $p = 0.19$, Faith's PD, $p = 0.70$). The beta diversity of the uterus 8 days after ovulation differed based on the embryo outcome, in both mares bred to the donkey and the horse ($R2 = 0.069$; F-value = 2.01; $p = 0.02$). These results revealed that seminal microbiome affected uterine microbiome and suggested for the first time in the horse that there is a temporary combined male and female microbiome with consequences on physiological reproductive functions like the establishment and development of an early pregnancy.

Keywords: Interspecies breeding, donkey microbiome

Glucuronide estrone and estradiol secretion in pregnant mare serum: insight from a liquid chromatography tandem mass spectrometry analysis

Jérôme Ponthier,[a] Thomas Dubrowski,[b] Caroline Le Goff,[b] Stefan Deleuze,[a] Sophie Egyptien,[a] Etienne Cavalier,[b] Joy Ledecka[b]

[a]Equine Clinical Sciences Department, FARAH Comparative Veterinary Medicine,
[b]Department of Clinical Chemistry, CIRM, CHU de Liège, University of Liège, Liège, Belgium

The equine placenta produces conjugated estrogens: research mainly focused on sulfonated estrogens, although glucuronide estrogens were observed in early studies. These preliminary investigations were performed using immunoassays that lacked specificity for small molecules. However, the higher analytic accuracy of liquid chromatography tandem mass spectrometry (LC- MS/MS) enables to refine the knowledge about estrogens produced during equine pregnancy. This study aims to describe the evolution of estrone and estradiol glucuronide (E1G and E2G) during equine pregnancy in 2 breeds and to determine if their secretion is linked to native and sulfonated estrone or estradiol (E1, E1S or E2, E2S). Between 2020-2024, serum samples were collected monthly from 18 Warmbloods (WB) and 24 Spanish purebred (SPB) pregnant mares. From 4 months of pregnancy onward, the combined thickness of the uterus and placenta (CTUP) was measured by ultrasonography. Mares with enlarged, heterogeneous CTUP or clinical signs of placentitis pre or postpartum (premature lactation, vulvar discharge, abortion, abnormal macroscopic placenta, foal weakness) were excluded from this study. A dedicated LC-MS/MS method was used to assay serum E1, E2, E1S, E2S, E1G, and E2G. For the 93 pregnancies included, monthly hormone changes were assessed using the Kruskal-Wallis test, and potential concentration differences between breeds were studied with the Mann-Whitney test. Nonparametric Spearman's tests were used to assess correlations between E1G, E2G, E1, E2, E1S, and E2S. Data were reported as median (25th percentile, 75th percentile) with significance set at $p < 0.05$. The peak concentration of E1G (12,068 (6,53015,751) pg/ml) was observed at 2 months, whereas the peak value of E2G was lower (1,692 (1,0362,000) pg/ml) and observed at 4 months. There was a breed effect for both hormones, but profiles differed; E1G concentrations were higher in WB from 6-10 months of pregnancy, whereas higher E2G concentrations were observed in SPB at 3, 7, and 8 months. Concentrations of E1G were poorly correlated with other studied estrogens, with its strongest correlation observed with E1S ($r = 0.47$, $p < 0.0001$). Positive correlations were observed between E2G and E1 ($r = 0.66$, $p < 0.0001$), E1S

(r = 0.71, p < 0.0001), and E2S (r = 0.70, p < 0.0001). To the best of our knowledge, LC-MS/MS was never used to describe E1G and E2G kinetics during pregnancy, and differences in their concentrations between breeds of different sizes were not reported. Although glucuronosyltransferase genes are constantly expressed by the endometrium during pregnancy, maximum E2G concentrations were observed at 4 months, before the E1, E2, and E1S peaks at 5 months. Surprisingly, the E1G peak occurred earlier in pregnancy, 1 month after the onset of eCG production by endometrial cups, which has an FSH-like effect normally promoting follicular E2 secretion. This unique E1G kinetic during pregnancy was confirmed by the absence of correlation with E1, E1S, E2G, and E2S, which all correlated, suggesting different pathways of production and effects of E1G. Further studies should confirm the origin of early E1G production in pregnant mares and physiological effects of its secretion after implantation.

Keywords: Liquid chromatography tandem mass spectrometry, sulfonated estrogens, glucuronide estrogens, mare, pregnancy, placenta

Follicular dynamics in insulin resistant mares

Julio Prado,[a] Eduardo Prado,[b] Lannett Edwards,[c] Neal Schrick,[c] Craig Reinemeyer,[d] Tulio Prado[b]

[a]Axiota Animal Health, Fort Collins, CO, USA
[b]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA
[c]Department of Animal Science Institute of Agriculture, University of Tennessee, Knoxville, TN, USA
[d]East Tennessee Clinical Research, Rockwood, TN, USA

Obesity and insulin resistance have been linked to prolonged interovulatory period, aberrations in the estrous cycle, and continuous reproductive activity during the nonbreeding season. Equine metabolic syndrome has been determined to influence the intrafollicular environment of mare ovaries. In humans, insulin resistance has been linked to polycystic ovaries as part of polycystic ovarian syndrome. A study was conducted to determine 1. the impact of insulin resistance on follicle growth and size at ovulation, and 2. whether predicted ovulatory follicles respond to human chorionic gonadotropin (hCG) treatment in insulin resistant (IR) mares. Mares were selected for the study based on insulin sensitivity (IS) and separated into an IR group (n = 6) and an IS group (n = 6). The ovaries and uterus were examined via transrectal ultrasonography at regular intervals during a spontaneous cycle and a $PGF_{2\alpha}$ shortened synchronized cycle. The dominant follicles (F1) had similar size and F1 size at ovulation between groups. There were more subordinate follicles in IR than IS mares (p < 0.05). The second largest follicle (F2) of IR mares was larger in diameter (p < 0.05) than the F2 of IS mares, which may signify a lack of dominance by the largest follicle. Treatment with hCG induced ovulation before 48 hours in 2 out of 4 IR mares, whereas 4 out of 4 in IS mares, although difference in time to ovulation after hCG treatment did not differ statistically. Results observed in this study may provide caution to practitioners working with IR mares with regards to numbers and sizes of secondary follicles and the effectiveness of hCG for induction of ovulation. The results of this study may support information that the mare could be used as a model to study human ovarian pathologies.

Keywords: Follicle, dynamic, insulin resistance, mare

Postmortem ovary harvest following intrathecal lidocaine hydrochloride injection in a mare

Valentine Prie,[a] Weston Davis,[a] Melissa Prell,[b] Justin McNaughten[a]

[a]Palm Beach Equine Clinic, Wellington, FL, USA
[b]Peterson and Smith Equine Hospital, Ocala, FL, USA

In the event of sudden death or euthanasia, postmortem ovary harvest and oocyte retrieval for in vitro embryo production affords owners a final opportunity to preserve a mare's genetic potential. In vitro embryo production can be affected by numerous factors including, but not limited to, season, intrinsic mare effects, pharmaceuticals, and ovary transit times. Researchers demonstrated that oocyte exposure to pentobarbital may negatively impact stages of in vitro embryo production.[1] In an emergency, many factors are beyond a practitioner's control; however, the method of euthanasia, surgical technique, and proper handling of the ovaries can be managed. We describe outcomes following euthanasia via intrathecal lidocaine hydrochloride injection as an alternative to an overdose of intravenous barbiturates. A 9-year, Warmblood mare, was presented for refractory septic tenosynovitis with extensive necrosis of the superficial and deep digital flexor tendons of the left hind limb. Despite heroic efforts, the owner elected to humanely euthanize the mare and requested postmortem ovarian harvest for oocyte retrieval and in vitro embryo production. Mare underwent general anesthesia; once in lateral recumbency, poll was clipped and a 4 inch spinal needle was inserted into the subarachnoid space, at the atlanto-occipital level. Intrathecally lidocaine hydrochloride 2% (dosed at 3.6 mg/kg) was given. After mare's death, a midline celiotomy was performed and ovaries were removed aseptically. Ovaries were processed, packaged, shipped, and oocytes were recovered as described.[2] A total of 22 oocytes were recovered; 11 matured oocytes were subjected to intracytoplasmic sperm injection with frozen- thawed sperm. Nine fertilized oocytes cleaved, resulting in 7 blastocysts that were vitrified. Maturation, cleavage, and blastocyst rates were 50, 81, and 64% respectively, comparable to commercial standards for in vitro laboratories in the US; however, they exceed rates published for oocytes recovered from postmortem ovary harvest.[3] In cases such as this, effective planning, procedural preparation, and transport coordination are crucial to maximize results when attempting genetic preservation. Successful execution of these elements can significantly enhance the chances of reaching the transferable blastocyst stage. In this case, the use of intrathecal lidocaine injection for euthanasia successfully produced multiple transferable blastocysts. Further research is necessary to compare this method of euthanasia to others and the effects on in vitro embryo production.

Keywords: Ovaries, oocyte, lidocaine, intrathecal, euthanasia, intracytoplasmic sperm injection

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Effect of follicle stimulating hormone commercial source and in vitro maturation medium formulation on equine oocyte maturation, cleavage, and blastocyst rates after intracytoplasmic sperm injection

Luisa Ramírez-Agámez, Charles Love, Camilo Hernández-Avilés

Equine Fertility Laboratory, Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

In vitro production of equine embryos by intracytoplasmic sperm injection (ICSI) of in vitro matured oocytes is a customary procedure in the equine breeding industry. Media utilized to induce oocyte in vitro maturation (IVM) is supplemented with hormones, particularly follicle stimulating hormone (FSH),[1] or combinations of FSH, luteinizing hormone (LH), and estrogens.[2] To date, comparisons among different commercial sources of FSH or IVM medium formulation on the efficiency of ICSI in horses are scarce. In this study, we sought to determine whether differences in the commercial source of FSH or the IVM medium formulation would impact the efficiency of ICSI in equine oocytes. In Experiment 1, 2 commercial sources of porcine FSH (Sioux Biochemical [SI] versus Prospec [PRC]; 5 mU/ml) added to a Medium 199 (M199)-based IVM medium were compared. In Experiment 2, 2 IVM media formulations (M199-FSH versus FSH + LH-ready-to-use commercial medium [IVFBS]) were compared. For all experiments, cumulus oocyte complexes (COCs) were recovered via transvaginal oocyte aspiration from 13 mares, aged 8-17 years, held overnight (12-18 hours) at 22°C, and then incubated in a humidified 5% CO2 atmosphere at 38.2°C for 30 hours. In vitro matured oocytes were fertilized by Piezo-driven ICSI using frozen/thawed sperm from a single fertile stallion, presumptive zygotes were cultured from days 0-5 at 38.2°C in a 5% CO2/6% N2 humidified atmosphere, and cleaved embryos (> 8 blastomeres) were further cultured for up to 5 more days in a 5% CO2/6% N2 humidified atmosphere. On days 7-10 after ICSI, blastocyst development was recorded. Differences in IVM, cleavage, and blastocyst rates per injected oocyte were compared by Fisher's exact test (JMP Pro 17). In Experiment 1, 191 follicles were aspirated and 121 COCs were recovered (SI [n = 68]; PRC [n = 53]). In vitro maturation (59% [n = 40] versus 60% [n = 32]), cleavage (65% [n = 26] versus 63% [n = 20]), and blastocyst rates (35% [n = 14] versus 34% [n = 11]) were similar for SI and PRC, respectively ($p > 0.05$). In Experiment 2, 353 follicles were aspirated and 226 COCs were recovered (M199-FSH [n = 113]; IVFBS [n = 113]). In vitro maturation (52% [n = 59] versus 61% [n = 69]), cleavage (64% [n = 38] versus 74% [n = 51]), and blastocyst rates (31% [n = 18] versus 25% [n = 17]) were similar for M199-FSH and IVFBS, respectively ($p > 0.05$). Overall, the results of this study indicated that 2 commercial sources of FSH (SI versus PRC), and 2 IVM medium formulations (M199-FSH versus FSH + LH ready-to-use commercial medium [IVFBS]) yielded similar oocyte maturation, cleavage, and blastocyst rates following ICSI. These results provided helpful information for ICSI laboratories regarding some factors that may or may not affect the efficiency of ICSI in horses.

Keywords: Horse, oocyte, intracytoplasmic sperm injection, in vitro maturation

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Severe uterine torsion and moderate anemia in a late-term pregnant Maine Coon

Leah Ramsaran, Soon Hon Cheong, Elizabeth Ippolito Hines, Alyssa Chandler, Christian Folk, Brian Chambers, Gerald Duhamel, Callum Donnelly

Department of Clinical Sciences, Cornell University, College of Veterinary Medicine, Ithaca, NY, USA

An 18-month, female Maine Coon cat (~ 55 days pregnant), was presented to a referral emergency service with signs of lethargy. Pale mucous membranes were noted at time of admission. Initial bloodwork revealed moderate, normocytic hypochromic regenerative anemia (HCT 18%, MCHC 31 g/dl, MCV 49 fl, absolute reticulocyte 185 thousand/µl), thrombocytopenia (88,000/µl), stress leukogram (segmented neutrophils 13,000/µl, lymphocytes 800/µl, eosinophil 0/µl), total hypocalcemia (8.1 mg/dl), and elevated AST (123 U/l). Patient was Triple SNAP (Idexx) negative, and no hemoparasites were identified on blood smear evaluation. Ultrasonography revealed 2 deceased fetuses, 2 live fetuses with heart rates between 220-240 beats per minute, and suspected placental separation. Given the poor prognosis for litter survival, the owners elected for pregnancy termination and ovariohysterectomy. At surgery, a 720-degree uterine torsion at the level of bifurcation and 2 jejunal intussusceptions were identified. En bloc hysterectomy and intussusception reduction were performed without incident. Histopathology confirmed uterine torsion with transmural hemorrhage and necrosis in the affected horn. Amniotic fluid and meconium aspiration were documented in the fetuses, indicative of fetal hypoxia. Aerobic and anaerobic cultures of uterine fluid were negative, and hemotropic Mycoplasma PCR was undetected. Postoperative management included 1 blood transfusion, antibiotic therapy, antiemetics and pain management. The queen recovered uneventfully and was discharged 2 days after presentation. This case demonstrated the vague clinical presentation of severe uterine torsion in cats. Although uterine torsion is a rare complication during feline pregnancy, blood sequestration within the torsional uterine horn should be included when considering differentials for anemia in late-pregnant cats.

Keywords: Cat, uterine torsion, anemia, intussusception

Schistosomus reflexus as a cause of cesarian surgery in a dog

Tressa Reiner,[a] Theresa Beachler,[b] Megan Bernard,[b,c] Olufemi Fasina[d]

[a]Department of Veterinary Clinical Sciences, [b]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[c]Genesee Valley Equine Clinic, Scottsville, NY, USA
[d]Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

Schistosomus reflexus (SR) is an infrequent but lethal congenital anomaly most common in cattle (0.01-1.3% of dystocias) but also reported in sheep goats, pigs, donkeys, horses, dogs, cats, and several exotic species.[1] The condition is characterized by ventral curvature of thoracic vertebrae (reflexus) and exposed abdominal and even thoracic viscera (schistosomus). A 4-year Labrador Retriever dog was presented for dystocia after successfully whelping 5 live pups. On reproductive ultrasonography 3 fetuses were observed with the caudal most fetus in the birth canal displaying signs of fetal distress with a heart rate of 130 beats per minute (bpm) while the other 2 had normal heart rates above 180 bpm. No fetus or fetal membranes were palpable in the vestibule. It was elected to proceed to emergency cesarian surgery. Two live pups were delivered from the uterine horns. The last pup was obstructed in the uterine body, identified in dorsal transverse presentation, and noted to have severe congenital abnormalities consistent with SR. Gross abnormalities of the pup included: a severe ventral abdominal wall defect with intestinal, hepatic, gastric, and splenic herniation, severe vertebral kyphosis and left ventricular cardiomyopathy. To date the etiology and embryological mechanism by which SR develops is unknown, although genetic abnormalities are suspected as the primary cause. Several genes associated with midline defects have been reported in mice[2] and in humans with thoracoabdominal syndrome, a syndrome similar to SR, an X linked gene has been reported.[3] Further studies are needed to better elucidate the etiology of SR.

Keywords: Dog, schistosomus refluxus, dystocia, cesarian surgery

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Adhesion induced pyometra and subsequent peritonitis in a Miniature Horse

Mikayla Reynaud, Alexandra Grillos, Marco Coutinho da Silva, Erin Runcan

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

Pyometra is an infection of the uterus with accumulation of purulent fluid often due to compromise of the cervix and hindered uterine clearance. Pyometra can lead to infertility, but rarely leads to clinical signs of disease in the mare where many cases can be manually drained and medically managed.[1] A 7-year, miniature horse mare, was presented for evaluation of a corneal ulcer and malodorous vaginal discharge. Transabdominal ultrasonography revealed a distended uterus filled with highly cellular, echogenic fluid. Transrectal palpation revealed a firm, extremely distended uterus with nonpalpable ovaries. Vaginal speculum examination revealed purulent discharge in the caudal vagina and what appeared to be a persistent hymen with an 'os' in the center, and purulent fluid draining from it. Cervix was not palpable. To permit drainage, the vaginal defect was manually dilated, and revealed distinct communication with the peritoneal cavity. There were multiple adhesions covering the opening of the cervix, interfering with drainage of the uterus. Mare began destabilizing the next day and was rushed into emergency surgery for an ovariohysterectomy. Multiple focal adhesions, inflammation of the peritoneum, necrotic tissue and a prior ventral midline surgical incision were noticed during surgery. Uterus was remarkably enlarged and drained prior to removal. Mare's abdomen was closed, and the mare was placed in the Trendelenburg position, to allow for access to repair the vaginal laceration. After several surgical and postsurgical abdominal lavages, the mare recovered uneventfully. It was concluded that the mare had undergone an undisclosed trauma to cranial vagina, potentially a dystocia, that led to a permanent communication between vagina and peritoneum. Although the mare had been able to adapt to this condition over time, it was likely that peritonitis was imminent without this discovery by the reproductive service.

Keywords: Pyometra, cervix, adhesions, ovariohysterectomy, peritonitis

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Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723

Comparison of the effect of motility stimulants on frozen-thawed semen in stallions

Leslie Rosales, Rebeca Rivera, Matheus Felix, Katrin Hinrichs

Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA, USA

Intracytoplasmic sperm injection (ICSI) can be used to produce equine embryos from low quality sperm. However, some sperm are of such low quality that identifying a motile sperm for injection can be difficult. Pentoxifylline (PTX) has a positive effect on motility of frozen-thawed sperm,[1] and the combination of penicillamine, hypotaurine, and epinephrine (PHE) has supported longevity of motility in sperm preincubated for IVF.[2] This study was performed to compare the effects of PTX and PHE on total and progressive motility of both good-quality semen (1 x frozen) and in a model of poor-quality semen (semen frozen and thawed 4 times, 4x frozen). Our aim was to identify a method to stimulate sperm motility for ease of selection during ICSI. Semen from each of 3 stallions was frozen in MFR5 commercial extender (E-Z Freezin – MFR5; Animal Reproduction Systems, Ontario CA, USA). A subset of the straws from each ejaculate were thawed and refrozen 3 additional times to produce 4x frozen straws. For each replicate, 1x and 4x straws were thawed and diluted with a modified Hank's balanced salt solution containing the following treatments: 1. no additives (Control); 2. with 2 mg/ml PTX; 3. with 4 mg/ml PTX; 4. with 1x PHE (9 mM hypotaurine, 18 mM penicillamine, and 1.8 mM epinephrine, the concentrations used for IVF2); or 5. with 2x PHE (twice the concentration as for 1x PHE). After centrifugation, the pellet was resuspended in the corresponding medium to 50 x 10^6 sperm/ml. A sample was assessed by computer assisted sperm analysis to determine total motility (TMOT) and progressive motility (PMOT) immediately after resuspension (T0) and after holding at 38°C in air for 30 minutes, and 1 and 2 hours. Data were analyzed using the Kruskal-Wallis test with pairwise Wilcoxon tests, with a significance of $p < 0.05$. Initial TMOT and PMOT for 1 x semen were 28.0 ± 4.9 and 17.2 ± 3.3 (mean ± SEM), respectively; these values for 4x semen were 1.9 ± 0.5 and 1.1 ± 0.3, respectively. Treatment with PHE at either concentration had no significant effect on TMOT or PMOT at any time in either 1x or 4x semen. In contrast, for 1x semen, treatment with PTX2 and PTX4 significantly increased PMOT at T0 and T30. For 4x semen, treatment with PTX2 and PTX4 significantly increased both TMOT and PMOT at essentially all time points; for example, at T30, PMOT for 4x semen in the Control and PTX4 treatments were 0.18 ± 0.1 and 2.6 ± 0.5, respectively ($p < 0.0001$). We conclude that PTX at either of the tested doses increased TMOT and PMOT in poor quality frozen-thawed semen and may have application during selection of sperm from poor quality semen for ICSI. In contrast, PHE had no effect on sperm TMOT or PMOT under tested conditions.

Keywords: Sperm motility, motility stimulants, low quality semen, pentoxifylline, epinephrine

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Chronic endometritis in a Thoroughbred mare with *Bordetella bronchiseptica*

Hayley Rossiano,[a] Brittany Middlebrooks,[a] Kristina Lu,[a] Karen Wolfsdorf,[a] Tina Elam,[a] Martha Brown,[a] Monica Martins Marcuzzi,[b] Emma Adam,[c] Erdal Erol,[c] Tirth Uprety,[c] Luke Fallon[a]

[a]Hagyard Equine Medical Institute, Lexington, KY, USA
[b]Equine Therapy Inc, Lexington, KY, USA
[c]Department of Veterinary Science, University of Kentucky, Lexington, KY, USA

A 3-year, Thoroughbred mare, was presented for evaluation of subfertility with a history of chronic infectious endometritis. Throughout the breeding season, multiple samples including uterine swabs, low volume uterine lavage (LVL), and a uterine aspirate were submitted for aerobic culture and cytology, revealing growth of *Pseudomonas putida* (*P. putida*) and β-hemolytic *Streptococcus* spp. Despite intrauterine treatments based on antimicrobial sensitivity, no pregnancies were achieved over 2 estrous cycles. Evaluation of the mare's perineum revealed a vertically positioned vulva with two-thirds distal to the pelvic brim. A caslick was in place; however, the dorsal vulva and perineal body were markedly flaccid. Notable findings on transrectal ultrasonography included fluid throughout the uterine lumen and air present in the cranial vagina. Hysteroscopy showed inflammation of the endometrium with the right horn appearing to have more severe, chronic inflammation. A direct uterine fluid aspirate obtained via hysteroscopy was submitted for aerobic culture and cytology. N-acetylcysteine was infused. Ultrasonographic examination the following day revealed cloudy fluid with swirling debris. Uterine lavage was performed and submitted for aerobic culture and cytology. Cultures were consistent with *Bordetella bronchiseptica* (*B. bronchiseptica*). Due to the similarities between *P. putida* and *B. bronchiseptica*, both oxidase- positive and appearing as small, grey, mucoid colonies on agar plates, the samples were submitted for identification. The isolate was positively identified as *B. bronchiseptica* via Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry (MS). After reviewing the mare's historic culture results it was hypothesized that the previously identified *P. putida* samples were *B. bronchiseptica*. Treatment was aimed at correcting anatomical abnormalities and treating infectious endometritis. A Gadd procedure was performed. Intra-uterine ozone therapy utilized a combination of ozonated sterile saline lavage followed by insufflation of the uterus with ozone gas once

daily for 5 days while the mare was in estrus.[1] This alternative therapy was chosen due to the mare's history of receiving multiple intrauterine antibiotics. Four weeks later, a uterine swab yielded no bacterial growth on aerobic culture. Unfortunately, *B. bronchiseptica* was detected again several weeks later when the mare was cultured prior to breeding. Mare was treated systemically with chloramphenicol for 2 weeks, and repeat culture resulted in no bacterial growth. Cytology was collected and submitted for genomic mapping. Shotgun metagenomic analysis produced a low number of reads of *Bordetella* spp., confirming MALDI-TOF results but signaling low confidence that a substantial amount of the bacterium was present within this sample. At this time the mare is due to be bred this season. *B. bronchiseptica* is a gram-negative bacterium known as a respiratory disease-associated pathogen in various species, notably dogs and swine. Although detecting it in the uterus is rare, it has been reported twice; in a mare with infertility[2] and an aborted equine fetus.[3] This report adds to our knowledge of this uncommon occurrence. Given its similarities to *P. putida*, *Bordetella* infections may be underreported due to misclassification. This abstract highlighted the need for advanced diagnostics, such as MALDI-TOF MS, to improve accuracy in bacterial identification, essential for appropriate clinical management and treatment.

Keywords: *Bordetella bronchiseptica*, endometritis, mare, ozone, nonantibiotic

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Phenotypic variation in female caprine XX/XY hematopoietic chimeras

Adamarys Ruelas,[a] Alan Conley,[a] Casey Caruso,[a] Benjamin Rupchis,[b] Rebecca Bellone,[a] Fauna Smith[a]

[a]Population Health and Reproduction, School of Veterinary Medicine,
[b]Department of Animal Science, University of California, Davis, CA, USA

XX/XY hematopoietic chimerism is caused by the anastomosis of placental vasculature between male and female fetuses, resulting in the exchange of hematopoietic stem cells. This phenomenon is common in cattle, with bovine XX/XY blood chimeric females typically being infertile but, presenting with variability in gonadal and uterotubular development due to exposure to male factors. This case report describes 2 female, caprine XX/XY hematopoietic chimeras (A and B) characterized phenotypically through physical examination, hormonal assays, diagnostic assessment of the reproductive tract and pathology, and blood and tissue genotyping. Goat A, was born triplet to 2 male littermates and goat B, was born triplet to a female and male littermate. At birth, both presented as horned, Saanen female goats. At 4 months, Goat A developed secondary male sex characteristics, including an enlarged poll with long hair and a full beard, vulva with coarse, excessive hair and an enlarged clitoris, accompanied by unusually small, short, narrow teats and a blinded end vagina, approximately 4.5 cm in length. Goat A underwent a necropsy which revealed a short vagina, with no discernable uterus and 2 small gonads with a smooth homogenous surface. Both gonads were attached to a tubular structure curving around the gonad from the cranial poll and both were associated with a vascular plexus. Goat B appeared phenotypically unremarkable with no secondary male sex characteristics. A vaginal examination revealed a normal vaginal length of 15.4 cm and the visualization of the external cervical os. Goat B responded to an estrus synchronization protocol and underwent laparoscopic examination, artificial insemination and visualization of uterine horns, oviducts, and ovaries (had follicular activity). However, Goat B failed to conceive after insemination or natural mating on 6 subsequent cycles, although progesterone concentrations were consistent with corpus luteum formation. Antimüllerian hormone (AMH) and testosterone concentrations were measured from birth through puberty along with age-matched male (n = 7) and female (n = 6) herd mates. At birth, both goats had AMH concentrations like the male controls. After 3 weeks, Goat A had evidence of endogenous AMH production in the female range. After 7 days, Goat B had AMH concentrations that were at or below the limit of detection. Peripubertal testosterone concentrations of goat A were between the male and female control ranges, whereas goat B had testosterone concentrations in the female control range at all timepoints. XX/XY hematopoietic chimerism was confirmed by PCR for X and Y specific genes. Chimerism was confined to the hematopoietic compartment with DNA isolated from hair follicles being positive for only the X chromosome (based on AME genotyping), whereas blood was positive for Y chromosome genes, SRY and AME Y1, as well as AME on the X chromosome. Karyotyping revealed XX and XY lymphocytes in both animals. Additionally, microsatellite marker testing revealed some loci with 3 alleles identified in blood, but only 2 in hair, consistent with hematopoietic chimerism. This report demonstrated the variable phenotype of caprine XX/XY blood chimeras ranging from substantial masculinization of a female to infertility in an otherwise classic, phenotypic female.

Keywords: Goat, hematopoietic chimerism, antimüllerian hormone, disorder of sexual differentiation

Ovine fetal deformities due to Cache Valley virus in the Southeast

Jessica Rush, Dane Schwartz, Hannah Maxwell, Katelyn Waters

Department of Clinical Sciences, Auburn University College of Veterinary Medicine, AL, USA

Six multiparous Katahdin ewes were pasture bred in a controlled breeding season. Ewes were confirmed pregnant via ultrasonography at ~ 40 days after ram exposure; however,

60 days later, 3 of the ewes were diagnosed not pregnant. Three ewes underwent elective cesarian surgery. Prior to surgery, transabdominal ultrasonography confirmed that fetal pregnancy age was > 145 days. Two lambs were delivered from each ewe. First ewe had 1 normal and 1 nonviable lamb with severe limb and spinal deformities. Second ewe had 2 normal lambs; however, noteworthy fibrin was observed on the placenta. Third ewe had 2 nonviable deformed lambs with fibrin on the placenta. Abnormal fetoplacental units were submitted for necropsy that revealed systemic denervation atrophy of the muscle resulting in limb and spinal deformities, internal hydrocephaly, severe cerebellar hypoplasia, and spinal cord atrophy. Blood samples from ewes were submitted to Texas A & M diagnostic laboratory for Cache Valley virus neutralization. Virus neutralization revealed antibody titers ranging 1:32-1:512, indicating tested animals were positive. Cache Valley virus causes abortion in small ruminants infected ~ on day 30 of pregnancy. The congenital abnormalities identified in this case indicated infection ~ on days 32-37 of pregnancy. The 3 ewes that were nonpregnant were likely infected prior to day 30. Ewes infected after 37 days of pregnancy can give birth to normal offspring. This herd had no history of new additions to the herd in the last 9 months. The affected ewes were born and raised on farm, with no history of travel. The only known potential biosecurity breach is that this herd belonged to a teaching institution and is housed on the same property as a veterinary hospital. As an arthropod transmitted disease, animals adjacent to infected patients are at risk of infection. Cache Valley virus is known to cause these abnormalities; however, this is a virus typically observed in the Western US. To the authors' knowledge, the first reported case in the southeastern US, with the closest reported cases in Texas, Kentucky, and Virginia.[1,2]

Keywords: Cache Valley Fever, congenital abnormalities, vector

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Hemospermia secondary to cutaneous habronemiasis of the urethral process

Eleanor Sandt

University of Pennsylvania School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

In the summer of 2023, a 12-year, Welsh pony stallion, was diagnosed with hemospermia secondary to cutaneous habronemiasis of the urethral process. Habronemiasis is a parasitic skin condition in equids caused by Habronema larvae. Flies deposit larvae on the horse's skin, particularly around the prepuce and urethral process, leading to granulomatous skin lesions. The diagnosis in this stallion was confirmed via punch biopsy and histopathology. The initial treatment included ivermectin, sexual rest, and topical fly repellents. However, despite these measures, the stallion's urethral process remained mildly irritated and inflamed, and the hemospermia persisted into the 2024 breeding season. Further diagnostics, including urinalysis, ultrasonography of the accessory sex glands and scrotum, and endoscopic evaluation of the urethra and bladder, revealed no other underlying causes for the hemospermia. To obtain a blood-free ejaculate, manual semen collection was attempted. However, the stallion failed to ejaculate, and the inflamed tissue of the urethral process bled. An open-ended Missouri artificial vagina (AV) was subsequently used, allowing fractionation of the ejaculate and direct observation of the urethral process during ejaculation. Using this method, all 3 fractions of the ejaculate were free of blood. Additionally, the open-ended design of the AV minimized urethral trauma during collection. Semen was successfully collected throughout the 2024 breeding season using this technique. Despite the resolution of hemospermia, the urethral process remained inflamed. Steroid therapy was considered to address urethral inflammation but was ultimately avoided due to the stallion's elevated resting insulin concentrations and the associated risk of laminitis. Instead, the stallion received multiple rounds of ivermectin treatment and daily applications of topical fly repellents. After approximately 1 year, the urethral process fully healed.

Keywords: Hemospermia, habronemiasis, open-ended artificial vagina

Comparing vaginal douche, cervicovaginal mucus, and uterine lavage for diagnosis of *Tritrichomonas foetus* in naive heifers exposed to a naturally infected bull

Dane Schwartz,[a] Jennifer Koziol,[b] Alexis Thompson,[c] Jessica Klabnik,[a] Jessica Rush,[a] Chance Armstrong[a]

[a]Department of Clinical Science, Auburn University, Auburn, AL, USA
[b]School of Veterinary Medicine, Texas Tech, Amarillo, TX, USA
[c]Texas A&M Veterinary Medical Diagnostic Laboratory, Canyon, TX, USA

Trichomoniasis, caused by the obligate extracellular protozoan *Tritrichomonas foetus* (*T. foetus*), is a venereal disease of cattle. *T. foetus* enters the cow through coitus and establishes infection throughout the entire reproductive tract. Inflammation of the reproductive tract secondary to *T. foetus* infection commonly results in embryonic loss resulting in prolonged interestrus intervals, low pregnancy rates, lighter weaning weights, and increased culling of open cows. Consequently, there is little known about sampling for *T. foetus* in the female. To the authors' knowledge, this is the first critical evaluation into sampling various anatomical regions in the cow using real time polymerase chain reaction (qPCR). We hypothesized that: 1. Samples taken from the uterus are more often positive for *T. foetus* compared to samples from the vagina and cervix; and 2. the prevalence of positive samples decreases over time, regardless of sample location. In naive heifers exposed to a naturally infected bull, the objective of the study was to compare samples taken from vaginal douche, cervicovaginal mucus, and low-volume uterine lavage for diagnosis of *T. foetus* (using qPCR). Eleven 14-month,

crossbred virgin *Bos taurus* heifers were utilized in this study. Heifers were exposed for 30 days (September 24, 2024-October 24, 2024) to a 5-year, bull naturally infected with *T. foetus*. Starting 30 days after the introduction of the bull, 3 sampling periods were performed, 21 days apart (T1: October 24, 2024, T2: November 13, 2024, and T3: December 5, 2024). At each sampling period, samples were collected in sequence of ascending anatomy: vaginal douche, cervical mucus aspirate, and low-volume uterine lavage. At the conclusion of each sampling day, all samples were transported to the Texas A&M Veterinary Diagnostic Laboratory Canyon, Texas location. Samples were submitted for individual qPCR. A total of 82 samples taken from 11 heifers over 3 sampling time points (T1, T2, and T3) and 3 sampling locations (vaginal douche, cervical mucus aspirate, and low volume uterine lavage) were collected. Twelve out of the 82 samples (14.6%) were positive on qPCR. Time significantly impacted the PCR results ($p = 0.005$), but location did not ($p = 0.87$). There was no significant difference among the timepoints. The positive samples were from 2 heifers, at T1 and T2. For the 2 positive heifers, all 3 specimens were positive at each respective time point. The 2 positive heifers were subsequently negative for specimens collected at T3. A uterine sample was not obtained for 1 heifer at T3. All other heifers were negative at all time points. In summary, there was no significant difference in sampling location, but there was in time. Although a small study, data suggested that the location of sampling is not important, but that sampling should occur within 51 days after exposure for best outcomes.

Keywords: *Tritrichomonas foetus*, qPCR, diagnostics, heifer, protozoan

Development of a diagnostic test for nocardioform placentitis

Kirsten Scoggin,[a] Barbara Whitt,[a] Rebecca Ruby,[b] Allen Page,[a] Emma Adam,[a] Shavahn Louxa[c]

[a]Gluck Equine Research Center, [b]Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY, USA
[c]School of Animal Sciences, Louisiana State University, Baton Rouge, LA, USA

Nocardioform placentitis (NP) is a form of mucoid placentitis which results in episodic abortions in mares with few external signs of disease. NP is characterized as a mucoid placentitis in which the bacterial infection is limited to the chorionic surface of the placenta without infection of the fetus, with the ecology and biology of the major causative organisms, *Crossiella equi* and *Amycolatopsis* spp. remaining unknown. As NP is notoriously difficult to diagnose, we hypothesized that we could utilize the technology behind tuberculosis testing in humans to develop a sensitive and specific test for NP in horses. With this methodology, viable peripheral blood mononuclear cells (PBMCs) are extracted from heparinized whole blood, then plated on an equine interferon gamma enzyme-linked immunosorbent spot plate at 25×10^4 cells/well. Three wells were exposed to media plus Nocardioform-specific antigens (Ag) whereas another 3 were exposed media alone (Con). Mares were considered positive when their PBMCs had > 2-fold increase in the number of IFNy producing cells in Ag-treated wells with a $p < 0.05$. Fetal membranes were evaluated by the University of Kentucky Diagnostic Laboratory. In 2024, 30 mares were successfully evaluated, including 8 with NP. In 2025, 60 mares deemed 'at-risk' by farm managers were enrolled in a prospective study with monthly blood samples. An additional 15 mares have enrolled with suspected disease based on clinical signs or transabdominal ultrasonography results. In 2025, finalized diagnostic reports were available for 19 mares thus far, with the remaining 56 mares yet to foal. Across both 2024 and 2025, the test had 83.3% sensitivity and 88.37% specificity with 10 true positives, 38 true negatives, 5 false positives and 2 false negatives. Currently, work is underway to optimize the test and assess repeatability.

Keywords: Placentitis, nocardioform, mare, diagnostic test

Granulosa cell tumor in a pregnant dog

Alyssa Shelby,[a] Bruce Christensen[a], Alan Conley[b]

[a]Kokopelli Veterinary Center, Sacramento, CA, USA
[b]Population Health & Reproduction, College of Veterinary Medicine, University of California, Davis, CA, USA

A 6-year, multiparous Boston terrier dog bred via transcervical insemination (based on progesterone timing) was presented for transabdominal ultrasonography pregnancy evaluation. Dog was on day 32 after LH surge at ultrasonography and breeders reported no obvious changes in behavior. There were several resorption sites in both uterine horns and there was only 1 embryo with a heartbeat and development appropriate for day 32 after LH surge in the right uterine horn. Left ovary was noted to be enlarged (5.9 x 4.7 cm) and had cystic cavitations. The margin of the spleen was clearly identified, confirming the suspicion the mass was ovarian in origin. Serum from multiple days during estrus and pregnancy were submitted for antimüllerian hormone (AMH) concentrations, which were elevated, with some higher than the upper limits of the assay (> 12 ng/ml). Concern for a possible granulosa cell tumor (GCT) was high on the differential list and unilateral ovariectomy was performed. Histopathology confirmed the diagnosis of GCT with clean margins achieved. Pregnancy was carefully monitored via transabdominal ultrasonography, tocodynamometry, and serial progesterone concentrations throughout the pregnancy. Terbutaline dosed at roughly 0.05 mg/kg (1/4 of 2.5 mg tablet) was given as a tocolytic agent by mouth every 12 hours. On day 64 after LH surge, the dog was presented for elective cesarian surgery; a viable, healthy male neonate was delivered. Subsequent AMH concentrations were substantially lower; at 72 hours the concentrations were < 3 ng/ml). To the authors' knowledge, this is a unique case of a confirmed GCT during mid-pregnancy with removal of the ovary resulting in a viable neonate at the end of pregnancy. Based on this case, use of AMH as a diagnostic tool for canine GCT may prove useful and warrants further study.

Keywords: Granulosa cell tumor, ovarian tumor, ovariectomy, antimüllerian hormone

Presumed fetal anasarca dystocia in a Guernsey goat

Morgan Simpson, Katelyn Waters, Jessica Prim, Lily Lewis, Jessica Klabnik

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

A 2-year, 40 kg, Guernsey doe, was presented on emergency for dystocia. The doe was in stage 2 labor and was not progressing; manual delivery was attempted by owners with no success. On the previous day, another presumed anasarca fetus had been delivered on farm. Recommendation to not breed that doe or buck again were provided. The sire of the current dystocia was unknown. On initial examination, the doe was bright, alert and responsive, with visible abdominal contractions every 30 seconds to 1 minute. One edematous limb exteriorized to the level of the fetal carpus was protruding from the vulva. Vaginal palpation revealed an abnormal fetus with edematous front extremities, and an edematous body. Based on these examination findings, a presumptive diagnosis of fetal anasarca was made. Treatment options provided included cesarian surgery or fetotomy, with the latter selected. The doe received intramuscular oxytocin (10 units) and a lumbosacral epidural with 2% lidocaine (1 ml/20 kg). The abnormal fetus was successfully removed in 2 fetotomy cuts, with 1 normal kid delivered after removal. The live doeling was provided with colostrum replacer (at 15% of body weight) and was clinically normal on examination. The doe received subcutaneous oxytetracycline (20 mg/kg once) and meloxicam (1 mg/kg once). This case highlighted the importance of client education surrounding breeding animals that have potential genetic conditions. Due to the series of suspect anasarca kids on this farm, the genetic component could be presumed to be related to the buck. It is important to note the infrequency of this genetic anomaly reported within the caprine species.

Keywords: Edematous fetus, anasarca, fetotomy, dystocia, goat

Physiological response to estradiol cypionate-sulpiride treatment to seasonally noncycling mares

Jenny Sones,[a] Isabella Faulkner,[a] Jennifer Hatzel,[a] Sarah Ward,[a] Patricio Razquin,[a] Javier Funes,[a] Brody Klinglesmith,[a] Pat McCue,[a] Erin Oberhaus[b]

[a]Equine Reproduction Laboratory, Colorado State University, Fort Collins, CO, USA
[b]School of Animal Sciences, Louisiana State University, Baton Rouge, LA, USA

Advancing the first ovulation of the year is a rate limiting step in equine breeding programs. Artificial light is the most reliable method but can be challenging for large groups of mares maintained outside. Typically, this requires mares being under artificial lighting ~ for 60 days. Therefore, an alternative method requiring less time to hasten ovulation in the deep anestrus (DA) mare is desirable. Sulpiride treatment, a dopamine antagonist, in estrogen-primed DA mares increased prolactin and luteinizing hormone concentrations. We hypothesized that DA mares given estradiol cypionate (ECP) and sulpiride ovulate earlier than vehicle-treated mares. Beginning in January, 12 mares (10-19 years) housed in northern hemisphere paddocks without artificial lighting were enrolled in a crossover study. At the start of the study, the largest median follicle size was 14.5 mm (range: 8-26 mm). Mares were randomized and treated with intramuscular ECP (50 mg) on day -1 and intramuscular sulpiride (3 mg) on day 0 and vehicle (n = 6/group). Transrectal ultrasonography was performed every other day until a 30 mm follicle was detected with uterine edema, then every day until ovulation. Serum progesterone concentrations were measured on days 5 and 12-14 after ovulation. Using a two-way ANOVA, a statistically significant difference in follicle size was observed by day 7 (treatment: 24 ± 2 mm; vehicle: 17 ± 2 mm). All treated mares developed a 30-35 mm follicle with uterine edema between 7-12 days, whereas none of the vehicle-treated mares exhibited follicular development (< 30 mm). Treated mares continued to cycle and therefore were not used as controls in the crossover. Six control mares were then used for a total of 12 ECP-sulpiride treated mares from January-February. These mares developed a 30-35 mm follicle with uterine edema between 11-18 days. Ten of the 12 total treated mares developed a follicle ≥ 35 mm and uterine edema (11.4 ± 1.5 days). One mare spontaneously ovulated, whereas 71.4% of the remaining mares ovulated after an ovulation induction agent. One mare developed a hemorrhagic anovulatory follicle. On days 5 and 12-14 after ovulation, serum progesterone concentrations of ovulated mares were 7.7 ± 1.02 and 7.9 ± 0.3 ng/ml, respectively. Treatment of noncycling mares with ECP-sulpiride significantly hastened follicle development and ovulation in this population of mares. This hormone combination may be clinically useful in both individual mares and large groups of embryo recipient mares.

Keywords: Mare, season, sulpiride, prolactin

Metritis in a postpartum mare associated with a retained hippomane

Reagan Stephens

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

Toxic metritis is an important condition in postpartum mares, commonly associated with retention of fetal membranes.[1] A 23-year, Quarter Horse mare, was presented 24 hours after dystocia with a sick foal. On presentation, the mare was normal except for mild trauma on the ventral vulva associated to dystocia and obstetric manipulation. Owners reported that the mare had passed her fetal membranes. The day after admission, the mare was lethargic, uncomfortable, had slight tachycardia (52 beats per minute), and low-grade fever (102.3°F). Transabdominal and transrectal ultrasonography were performed. Transabdominal ultrasonography identified minimal free fluid in the abdomen and a distended bladder. Also, a hyperechoic, elliptical structure consistent with a hippomane was floating in the uterine fluid.[2] There was no internal or external evidence of retained fetal membranes. The hippomane required manual removal since it would not pass though the lavage tube. The recovered uterine fluid, of brown-red discoloration, was examined by cytology (numerous neutrophils and rod-shaped bacteria) and culture (pure growth of Escherichia coli after 24 hours of

culture). Follow-up treatments included antibiotics, anti-inflammatories, oxytocin, and lavage until minimal uterine free fluid and no evidence of bacteria was observed. Of clinical relevance, the hippomane was not identified via transrectal ultrasonography, due to the limitation in the scanning depth of the ultrasound but was only identified following transabdominal ultrasonography. In addition, if the hippomane had not been identified prior to lavage, it is unlikely that the hippomane, due to its size, would have passed through a lavage tube, and would have been retained in the uterus. Toxic metritis is commonly caused by retained fetal membranes and can lead to laminitis and death. This case is unusual since a retained hippomane, not retained fetal membranes, was associated with the toxic metritis.

Keywords: Horse, hippomane, metritis

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Effect of parturition induction methods on delivery of cloned lambs

Rusty Stott, Ying Liu, Cheyenne Marriot, Tayler Patrick, Renata Blocher, Misha Regouski, Iuri Perisse, Irina Polejaeva

Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA

Somatic cell nuclear transfer (SCNT) is a valuable tool in both medical and agricultural research, used to produce animal models for human diseases and domestic animals with high genetic value for breeding purposes. However, the low efficiency of SCNT hampers its application, with only 5- 15% of transferred embryos resulting in live births in domestic animals. One of the reasons is pre and postnatal abnormalities, such as prolonged pregnancy, increased birth weight, and reduced neonatal survival. Aim of this study was to investigate different induction protocols to improve the birth and survival rates of cloned lambs. Sheep SCNT embryos were generated using our standard protocol, and recipient synchronization and embryo transfers were conducted as described. On average, 14.9 ± 2.7 one-cell stage embryos were transferred into the oviduct of each synchronized recipient that were in estrus within 12 hours of the transfer time. The status of dominant follicle size or ovulation was evaluated before embryos were transferred into a recipient. Initial pregnancies were confirmed on day 40 ± 5 of pregnancy via transabdominal ultrasonography. Two protocols were used for parturition induction. Intramuscular dexamethasone (15 mg) was given once 24 hours before cesarian surgery on day 148 of pregnancy (short protocol). Intramuscular triamcinolone (2 mg) and tulathromycin (2.5 mg/kg) was given once on day 142 of pregnancy followed by dexamethasone (15 mg) and intramuscular cloprostenol (250 µg) given once 48 hours before cesarian surgery on day 148 of pregnancy (long protocol). After delivery, the offspring remained with their dams and nursed freely until weaning at 2.5-3 months of age. Delivery methods and survival rates were analyzed using a generalized mixed model, and birth weight and days of pregnancy were analyzed using a mixed model, with oocyte source, donor cell sex, recipient surgical history, and twin or singleton status as random effects (Jamovi software, version 2.6.44). $P < 0.05$ was considered statistically significant. In total, 3109 SCNT embryos were transferred into 210 recipients, with an initial pregnancy rate of 39.5% (83 out of 210). Four pregnancies were terminated for sample collection. Of the remaining 206 recipients, 52 (25.2%) went to term, with 14 animals following the short protocol and 38 animals following the long protocol. Compared to the short protocol, the long protocol resulted in higher rate of natural delivery and a lower rate of cesarian surgery births (7.1 and 92.9% versus 71.1 and 28.9%, respectively; $p = 0.004$). Duration of pregnancy was shorter for the long protocol than that for the short protocol (148 ± 1.9 versus 150 ± 1.5 days, $p = 0.010$), but birth weights did not differ between protocols (short: 7.79 ± 2.15 kg; long 6.63 ± 1.80 kg; $p = 0.588$). Moreover, survival rate of lambs at birth and at 1 month did not differ between the 2 protocols (short: 94.1% and 25%; long: 89.1% and 58.5%; $p = 0.928$ and $p = 0.718$, respectively). In conclusion, the long induction protocol improved the birth of cloned lambs through natural delivery, shortened pregnancy, and potentially benefitted their long-term survival.

Keywords: Parturition induction, cloned offspring, sheep

Extracellular vesicles secreted by mouse oviductal organoids: a model for contraceptive development

Riley Thompson,[a,b] **Ahmed Gad,**[a,b] **Mindy Meyers,**[a,b] **Fiona Hollinshead**[a,b]

[a]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA
[b]Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO, USA

Extracellular vesicles (EVs) are nanoparticles secreted by cells for intercellular communication via the transfer of bioactive molecules, such as lipids, proteins, and nucleic acids. Organoids are 3D, spherical cell clusters formed in vitro that are capable of long-term proliferation and self- organization with similar function to their tissue of origin. EVs produced by organoids are more similar to EVs produced in vivo than those produced in traditional 2D cell culture models because organoids retain more in vivo-like properties. The objectives of this study were to 1. generate mouse oviductal organoids using 2 culture conditions (static versus bioreactor) and 2. isolate and characterize EVs secreted by the mouse oviductal organoids. Our hypothesis was that EVs produced by mouse oviductal organoids share the same protein markers identified in EVs collected from the in vivo mouse oviduct: CD9 and Hsp70. Mouse oviductal cells (n = 3 mice) were used to generate organoids that were cultured for up to 189 days (passaged

every 7 days) either in a static organoid culture system or a dynamic CERO (Omni Life Sciences) bioreactor culture condition. Organoids were assessed via brightfield microscopy for maintenance of phenotype throughout the culture period. The spent culture medium from each culture condition was collected for isolation of EVs via differential ultracentrifugation. EVs were assessed for 1. size and concentration using nanoparticle tracking analysis (NTA), 2. morphology using transmission electron microscopy (TEM), and 3. the presence of EV-associated protein markers using a Jess Automated Western Blot system (Bio-techne). Brightfield imaging of mouse oviductal organoids demonstrated round cellular clusters with a central lumen for both static and bioreactor culture conditions, consistent with previous mouse oviductal organoid reports. EVs isolated from the static culture condition spent medium (starting volume = 20 ml) had a median diameter of 134 nm with a concentration of 9.4 x 10^9 particles/ml, and the EVs from the bioreactor culture condition spent medium (starting volume = 9.5 ml) had a median diameter of 159 nm diameter and a concentration of 13 x 10^9 particles/ml. Transmission electron microscopy demonstrated the characteristic 'cup-shaped' morphology for the EVs, and Jess analysis demonstrated the presence of EV proteins CD9 and Hsp70 and absence of the cellular contaminant protein CYCS. Data demonstrated that mouse oviductal organoid EVs express proteins expected in EVs secreted by mouse oviductal cells in vivo. Additionally, more EVs were produced per milliliter of spent medium in the bioreactor compared to the static culture condition; thus, the bioreactor appeared better suited for EV production from organoids. Future experiments will use mouse oviductal organoids to test novel contraceptives, such as EVs containing CRISPR-cas9 ribonucleoproteins that target essential genes for fertility, including PGR and OVGP1.

Research reported here was supported by the Office of the Director, National Institutes of Health under award number K01OD032455. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Keywords: Fallopian tube, 3D culture, murine, exosome

Vitamin D3 as a novel treatment for equine persistent breeding-induced endometritis

Jaden Thompson,[a] Kirsten Scoggin,[a] Sarah Barcalow,[b] Ashley Stark,[b] Whitney Woods,[b] Mats Troedsson,[a] Hossam El-Sheikh Ali,[a] Shavahn Louxa[c]

[a]Department of Veterinary Sciences, Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA
[b]Department of Veterinary Medicine, Lincoln Memorial University, Harrogate, TN, USA
[c]Department of Animal Sciences, Louisiana State University, Baton Rouge, LA, USA

Persistent breeding-induced endometritis (PBIE) is one of the leading causes of subfertility in mares, affecting 10-15% of the population. Mares susceptible to PBIE tend to be older animals with an impaired immune response, factors that are associated with vitamin D3 (D3) deficiency in humans. Previous research from our laboratory demonstrated a robust reduction in interval to clear uterine fluid and alterations in cytokine profiles suggestive of improved inflammatory response. We hypothesized that intrauterine D3 infusion into artificial insemination doses improves pregnancy rates and helps susceptible mares to clear fluid more effectively, with cytokine and transcriptomic profiles resembling more closely to resistant mares after D3 infusion. Forty-two mares were classified based on interval to uterine clearance, resistant (R; < 48 hours; n = 21) or susceptible (S; > 96 hours; n =13). Mares falling between these 2 thresholds were classified as intermediate and not considered for the remaining analyses. Mares were bred with 1 x 10^9 progressively motile sperm containing either D3 or vehicle (Con). Low volume uterine lavage (LVL), plasma and endometrial biopsies and samples were collected from mares 24 hours after breeding. LVL and plasma samples were analyzed for concentrations of 15 cytokines/chemokines using Milliplex Equine Magnetic Bead Panels (MilliporeSigma). Biopsies were used for RNA extraction and RNA sequencing (RNA-seq). Pregnancy was diagnosed on day 14 after ovulation. As in our earlier study, interval to clear fluid decreased ($p < 0.05$) in susceptible mares (Con – 7.75 ± 1.33 days; D3 2.66 ± 0.61 days;). Pregnancy rates (5/6; 83.3%) in susceptible mares improved ($p < 0.001$) with D3 treatment compared to susceptible mares treated with vehicle (0/8; 0%); although 1 D3 pregnancy was lost by 21 days of pregnancy. Pregnancy rates did not differ for resistant mares (Con = 4/8, 50%. D3 = 6/14, 43%). Cytokine profiles for LVL suggested D3 reversed trends observed in susceptible mares, with D3-treated mares having a decrease ($p < 0.10$) in IL-8, and an increase ($p < 0.01$) in IL-1α, with the susceptible D3 levels ultimately being indistinguishable from the control cycle of resistant mares. Other changes noted within susceptible D3-treated mares' plasma included an increase ($p < 0.1$) in IL-1α, decrease ($p < 0.05$) in G-CSF, and increase ($p < 0.05$) in eotaxin. These trends suggested an anti-inflammatory shift towards a more eosinophil-dominant, anti-inflammatory profile. Next-generation RNA-seq was performed using Illumina NovaSeq 6000. Differentially expressed genes (DEGs) were identified using JMP with a false discovery rate < 0.05. Our RNA-seq analysis revealed 78 genes differentially expressed between Con and D3 cycles in susceptible mares, with many of them corresponding to the genes differed between susceptible and resistant genes. Promisingly, D3 appeared to shift gene expression to mimic that observed in resistant mares. These included genes associated with the inflammatory response (MMP26), response to oxidative stress (PRKAA2), and VEGF signaling pathways (PIK3R1) among others. D3 has early promise to help alleviate PBIE in susceptible mares through improved interval to uterine clearance, improved cytokine response and improved pregnancy rates in susceptible mares.

Keywords: Horse, persistent breeding-induced endometritis, vitamin D3, uterine clearance, inflammation

Immunological and transcriptomic insights into equine persistent breeding-induced endometritis

Jaden Thompson,[a] Kirsten Scoggin,[a] Mats Troedsson,[a] Shavahn Loux,[a,b] Hossam El-Sheikh Ali[a]

[a]Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA
[b]School of Animal Sciences, Louisiana State University, Baton Rouge, LA, USA

Persistent breeding induced endometritis (PBIE) is a leading cause of subfertility in mares; however, the immunological and molecular consequences resulting

from postbreeding inflammation resolution failure are not fully understood. We hypothesized that characterizing cytokine concentrations in low volume lavage (LVL) and analyzing the endometrial transcriptome in PBIE-susceptible versus resistant mares elucidate key immunological and molecular outcomes associated with susceptibility to persistent inflammation. Twenty-two mares were categorized based on uterine fluid clearance time into resistant (R; ≤ 48 hours; n = 8), intermediate (I; 96 hours ≥ I > 48 hours; n = 6), or susceptible (S; > 96 hours; n = 8). Low volume uterine lavages (LVL) of 60 ml and endometrial biopsies were collected from all mares 24 hours after breeding. LVL samples were analyzed for the concentrations of 15 cytokines using Milliplex Equine Magnetic Bead Panels (MilliporeSigma). The biopsies were used for RNA extraction and RNA sequencing (RNA-seq). The first cycle pregnancy rate in susceptible mares was lower compared to intermediate ($p = 0.003$) and resistant ($p = 0.03$) mares. Luminex analysis revealed elevated ($p < 0.05$) concentrations of IL-5 and IL-6 in LVL samples from susceptible mares compared to intermediate and resistant mares. Additionally, IL-8 concentrations were elevated in LVL between susceptible mares and resistant mares ($p = 0.02$). Conversely, G-CSF, fractalkine, and IL-1α concentrations were decreased ($p < 0.05$) in LVL samples from susceptible mares compared to intermediate and resistant mares. Receiver operating characteristic (ROC) analysis was performed to evaluate the potential of cytokines as biomarkers for PBIE. IL-1α and G-CSF exhibited the highest accuracy ($p < 0.05$) in distinguishing susceptible mares from intermediate and resistant mares, with area under the curve (AUC) values of 0.86 and 0.84, respectively. Additionally, IL- 1α and G-CSF demonstrated the highest accuracy ($p < 0.05$) in predicting day 14 pregnancy status (pregnant versus nonpregnant) with AUC values of 0.83 and 0.84, respectively. Next generation RNA-seq was performed using NovaSeq 6000 (Illumina), and reads were mapped to EquCab3.0 (STAR-2.7.9a). Differentially expressed genes (DEGs) were identified using the DESeq2 package in R with a false discovery rate (FDR) < 0.05. RNA-seq analysis revealed 483 DEGs in the comparison of S versus R, 270 DEGs in S versus I, and 76 DEGs in I versus R mares. Gene ontology (GO) analysis of DEGs from the S versus R comparison revealed enrichment in relevant pathways, including inflammatory response (e.g. ODAM, CUL3, TAB2, CHI3L1, MMP26), response to oxidative stress (PRKAA2, MAPK8), nitric oxide biosynthesis (e.g. ASS1, GUCY1A1, AQP1), angiogenesis, and VEGF signaling pathways (e.g. HSPB2, FLT4, ARHGAP1, PIK3R1, SHC2), and negative regulation of muscle contraction (e.g. TPM2, LMOD1, ATP1A2), among others. These findings suggested that the identified pathways reflect downstream immunological consequences of PBIE, potentially contributing to delayed uterine clearance and reduced fertility. This study identified consequential markers associated with susceptibility and provided insight into the molecular mechanisms sustaining unresolved postbreeding inflammation. Together, these results may inform the development of targeted diagnostics and therapeutic strategies to improve outcomes in affected mares.

Keywords: Horse, persistent breeding induced endometritis, uterine clearance, inflammation, cytokines, transcriptome

Unilateral cryptorchidism with persistent paramesonephric duct remnants in a gelding

Joshua Trumble,[a] Erin Runcan,[a] Christopher Premanandan[b]

[a]Department of Veterinary Clinical Sciences,
[b]Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

A 3-year, Mustang gelding (gelded before purchase), was presented for aggressive behavior and mounting mares. On palpation, there were no testes and gelding had a distinct scrotal scar. Antimüllerian hormone concentrations were > 14 ng/ml (reference value: > 0.15 ng/ml in intact horses) and serum testosterone concentrations were 526.4 pg/ml (reference value: >100 pg/ml in cryptorchid horses) suggestive of gonadal tissue in the gelding. Palpation of the inguinal area under sedation revealed no palpable testis. Transrectal and transabdominal ultrasonographic examinations revealed a discreet mass resembling testicular parenchyma surrounded by a large amount of free, anechoic fluid. Due to the presumptive presence of retained testicular tissue, surgical excision was recommended. Gelding was anesthetized and placed in dorsal recumbency in Trendelenburg position for an exploratory laparotomy. On laparoscopic imaging, a large fluid- filled structure was identified on the right side surrounded by serosa that resembled testis. Ductus deferens and epididymis were traced from the structure to the ipsilateral ampulla. Yellow, serous fluid (650 ml) was aspirated from the structure to facilitate removal. The structure was ligated and excised unremarkably. On further exploration, the left ductus deferens was traced to and passed through the inguinal ring. The scrotal scar was explored and confirmed to contain no testicular tissue, consistent with hemicastration. Routine closure was performed, and gelding recovered uneventfully from anesthesia. On gross evaluation of the excised tissue, an additional 300 ml of fluid was removed from the structure. A firm, white mass with several cystic structures on its surface was identified within the larger cystic structure. The entire mass was submitted for histopathologic evaluation. Histopathology revealed atrophied seminiferous tubules and sustentacular cells devoid of spermatogenic epithelium within the firm mass. A larger cystic structure had regions of columnar epithelium and glandular structures resembling rudimentary endometrium. A diagnosis of a cryptorchid testis due to a testicular disorder of sexual development was made. During sexual development of the male, the paramesonephric duct typically regresses. Failure to regress results in structures such as uterus masculinus and appendix testis or hydatid of Morgagni that have not been previously described in horses.[1] This case highlighted a unique disorder of sexual differentiation in the stallion.

Keywords: Cryptorchid, horse, appendix testis, hydatid of Morgagni, disorder of sexual development

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Exploring the potential of equine endometrial organoids: tissue similarities, cycle-stage influence, and long-term stability

Margo Verstraete,[a,b] Fianne Kuijpers,[a] William Holl,[c] Machteld van Heule,[a,b] Jamie Norris,[a] Soledad Martin-Pelaez,[a] José Uribe-Salazar,[d] Christina Heard,[e] Anibal Armien,[e] Ward De Spiegelaere,[b] Mariano Carossino,[c] Peter Daels,[b] Pouya Dini[a]

[a]Department of Population Health and Reproduction, University of California, Davis, Davis, CA, USA
[b]Department of Morphology, Imaging, Orthopedics, Rehabilitation and Nutrition, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
[c]Department of Pathobiological Sciences & Louisiana Animal Disease Diagnostic Laboratory, Louisiana State University, Baton Rouge, LA, USA
[d]Genome Center, [e]California Animal Health & Food Safety Lab System, University of California, Davis, Davis, CA, USA

The endometrium is a dynamic tissue undergoing cyclic changes in response to ovarian hormones that are believed to be essential for reproductive success. Although endometrial organoids have been established as physiologically relevant in vitro models in various species, equine endometrial organoids (EEO) remain underexplored. This study aimed to characterize the structural and molecular properties of EEO and assess their fidelity to native endometrial tissue (END) across reproductive cycle stages and extended culture. We hypothesized that: 1. EEO and END exhibit a different transcriptome while retaining key endometrial markers; 2. organoid properties vary based on the reproductive cycle stage of the donor; and 3. most of the transcriptome remains stable over time. EEO were generated from endometrial biopsies recovered from mares in estrus and diestrus and the morphology, protein and gene expression were compared. In addition, transcriptomic stability of EEO was assessed across early (P2) and late (P15) passages. Histological evaluation demonstrated that EEO form cystic epithelial structures with a polarized columnar epithelium and basally located nuclei. Transmission electron microscopy (TEM) revealed the presence of microvilli, tight junctions, and secretory vesicles. Immunohistochemistry (IHC) confirmed the presence of epithelial (cytokeratin$^+$) and stromal (vimentin$^+$) cells in EEO. Gene expression overlap analysis revealed that 75.7% of genes were shared between EEO-P2 and END using bulk RNAseq, including key endometrial markers (FOXA2, MUC1, VIM, CD44, P19, ACP5, and SCGB1A1), at both bulk and single-cell RNA level. Paired transcriptomic analysis (FDR < 0.01) identified 1,331 genes upregulated in EEO-P2 compared to END, related to cell cycle, proliferation, metabolism, and reproduction, whereas 1,847 downregulated genes were linked to differentiation, immune responses, and vasculature development. TEM revealed significant differences in length and number of microvilli, and number of secretory granules per cell, between estrus and diestrus derived EEO. IHC confirmed estrogen receptor expression in estrus and diestrus derived EEO, whereas progesterone receptor expression was only detected in estrus-derived EEO. Bulk RNAseq identified 656, 1,705, and 185 differentially expressed genes (DEGs) when comparing estrus and diestrus in END, EEO-P2, and EEO-P15, respectively (FDR< 0.01). Genes associated with proliferation, secretion, and metabolism were upregulated in estrus derived EEO and pathways related to ciliary function and complement activation were upregulated in diestrus derived EEO. Transcriptomic stability analysis revealed that 65.6% of DEGs identified in EEO-P2 versus END overlapped with those in EEO-P15 versus END. These findings suggested a gradual loss of systemically regulated hormonal responses over prolonged culture, while cell-intrinsic traits persisted. To conclude, this study provided a comprehensive structural and molecular characterization of EEOs, demonstrating their potential as a physiologically relevant in vitro model for equine endometrial biology. By capturing aspects of reproductive cycle dynamics and maintaining key endometrial features over passages, EEO offer a valuable tool for investigating uterine physiology, intrauterine infections, and fetomaternal interactions, ultimately advancing equine reproductive research.

Keywords: Horse, endometrium, organoids, reproductive cycle

Granulosa cell tumor in a caprine ovotestis

Adam Ward,[a] Rachael Gately,[a] Robert Dwyer,[a] Fabio Rosa,[b] Carlos Pinto[a]

[a]Department of Ambulatory Medicine and Theriogenology, Cummings School of Veterinary Medicine, Tufts University, Grafton, MA, USA
[b]Department of Comparative Pathobiology, Cummings School of Veterinary Medicine, Tufts University, Grafton, MA, USA

A 3-year, Nubian goat with female external genitalia, was presented for evaluation of clitoral hyperplasia. Owners reported that the goat was born with a female twin, and displayed buck-like behavior, namely, mounting other goats associated with frequent Flehmen responses, and aggression as evidenced by excessive head butting and rearing around handlers. Additionally, the goat had the odor of a buck, attempted self-enurination, and possessed a beard and wattles. The goat appeared systemically healthy at presentation, and the only external abnormality noted was a hyperplastic and hyperpigmented clitoris that protruded from the vulva ~ 1 cm. Digital vaginal examination revealed a blind pouch with no external cervical os after vaginal examination with a speculum. Suspected gonads were identified by transabdominal ultrasonographic examination. The left gonad measured ~ 4 x 5 cm in diameter, and was associated with 2 cystic structures ~ 1 cm in diameter. The right gonad measured ~ 2 cm in diameter. The goat was sedated with intravenous ketamine (1 mg/kg), xylazine (0.05 mg/kg), and butorphanol (0.025 mg/kg), then placed in Trendelenburg position. Laparoscopic examination of the abdomen confirmed the abnormalities noted on ultrasonographic examination. Following intubation and maintenance of general anesthesia with isoflurane, the gonads were removed via a ventral midline laparotomy. The goat recovered uneventfully from anesthesia and received subcutaneous florfenicol (40 mg/kg), and intravenous flunixin meglumine (1.1 mg/kg). Histopathological examination of the gonads revealed tissue consistent with ovotestis and a granulosa cell tumor of the left ovotestis. Results from blood karyotyping were consistent with a disorder of sexual development, in that the goat was 80% genetically male 60, XY cells and 20% genetically female 60, XX cells. Molecular analysis revealed the goat was positive for the Y-linked SRY gene and positive for the X-linked androgen receptor gene. A recent search of the literature yielded no reports of a granulosa cell tumor in a caprine ovotestis.

Keywords: Ovotestis, granulosa cell tumor, os clitoris

Alpaca with a history of dystocia

Nathaniel Ward, Devynn Volding, Joanne Hardy, Mariah Pearson, Sophia Marchio, Yatta Boakari

Department of Large Animal Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX, USA

A 5-year alpaca, was presented for dystocia on day 366 of pregnancy. The alpaca had a history of 3 previous pregnancies that resulted in dystocia, with 1 cria surviving. Stage 1 labor began the morning before presentation but failed to progress over the next 9 hours. On admission, the alpaca was quiet, alert, and responsive, with normal vital parameters. Clinical examination revealed decreased gut sounds, mildly injected ocular conjunctiva, hyperglycemia, an elongated vulva, a relaxed sacrosciatic ligament and perineum, absence of abdominal contractions, and minimal cervical dilation. Transabdominal ultrasonography confirmed fetal death. To promote cervical dilation, 5 ml N-butylscopolammonium bromide was applied topically to the cervix. Additionally, 10 ml lidocaine 2% mixed with lubricant was given rectally to facilitate transrectal palpation and rule out uterine torsion, which was not diagnosed. Vaginal palpation revealed a fetus in a dorsopubic position with neck flexion. Following substantial obstetrical manipulation, a nonviable 9.98 kg fetus (average; 6.9-8.4 kg[1]) was delivered vaginally. The fetus was submitted for necropsy, and findings were unremarkable. Dystocia occurs in 2-5% of alpaca pregnancies, with < 1% requiring intervention.[2] Common maternal causes include uterine torsion, inertia, and cervical dilation failure.[2,3] Fetal causes primarily comprise of fetal malposition or congenital abnormalities.[3] Recurrent dystocia in this case suggested a maternal factor, though fetopelvic disproportion remains a consideration, given the cria's above average size and the need for obstetrical manipulation. Uterine inertia, secondary to prolonged stage 1 labor, and incomplete cervical dilation, further impeded parturition. Absence of cervical dilation has been noted as a cause of dystocia in alpacas, resembling a condition described in sheep as 'ring womb'.[4,5] This case illustrated the importance of timely obstetrical intervention during dystocia and supported the use of N-butylscopolammonium bromide to promote cervical dilation during parturition.

Keywords: Hembra, alpaca, camelid, dystocia, incomplete cervical dilation

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Management of unilaterally fixed twins in a multiparous American Quarter Horse mare

Baker White, Jessica Cowley, Rebecca Funk

Department of Large Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech University, Blacksburg, VA, USA

A multiparous, 13-year American Quarter Horse mare, was presented for reproductive evaluation 13 days after ovulation and breeding via artificial insemination. Transrectal ultrasonography revealed 2 embryos, positioned in the uterine body and uterine horn, respectively. Sedated manipulation and crushing of 1 embryo with the ultrasound probe were unsuccessful. On days 14-15 after ovulation, 4 additional examinations revealed close apposition of identically sized embryos that prohibited embryo reduction. During next 90 days, unilaterally fixed twins grew at similar rates and maintained heartbeats. On day 111 of pregnancy, fetal intracardiac injection of 1 twin was successfully performed at a referral hospital. Serial transabdominal ultrasonographic examinations in the following months revealed 1 viable fetus that was born without complications on day 348 of pregnancy. Placental evaluation revealed a mummified fetus invaginated within the live twin's placenta. Natural reduction of 1 twin occurs in 89% of unilaterally fixed twins with 82% of reductions occurring by 30 days of pregnancy.[1,2] However, when both embryos persist past 40 days of pregnancy, the likelihood of complete pregnancy loss is 63%.[3] With a 93% success rate, manual reduction of 1 embryo prior to fixation is the preferred twin reduction method.[4] Following fixation, multiple reduction procedures have been described, with varying success rates and pregnancy windows of utility. Examples of reduction procedures include transvaginal ultrasound- guided twin reduction and craniocervical dislocation.[5] Transcutaneous ultrasound-guided twin reduction, as performed in this mare, involves ultrasound-guided injection of potassium chloride or procaine penicillin directly into the fetal heart through the mare's abdomen on days 66-168 of pregnancy.[5] This case highlighted the utility of postfixation twin reduction techniques when embryo dynamics render preferred methods of prefixation reduction unsuccessful, even in a well- managed broodmare under the care of an experienced veterinarian.

Keywords: Horse, twin embryos, postfixation, reduction

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Effect of endometrial cyst removal via laser on pregnancy rates in the mare

Karen Wolfsdorf,[a] Carleigh Fedorka,[b] Kristina Lu,[a] Peyton Draheim,[a] Brittany Middlebrooks,[a] Hannah Vagedes[a]

[a]Hagyard Equine Medical Institute, Lexington, KY, USA
[b]Colorado State University, Fort Collins, CO, USA

Lymphatic and glandular cysts arise from collections of lymphatic fluid within the endometrium or myometrium, due to obstructed lymphatic channels or the gravitational effects of a pendulous uterus. The incidence of endometrial cysts in the general mare population has been reported as 1- 22%, with subfertile and older mares up to 55%. Endometrial cysts may reflect senility of the uterus by the correlation between the number of cysts and severity of pathological changes. The aim of this study was to determine the effect of endometrial cyst removal ablation via laser on pregnancy rates in barren/subfertile mares. Removal of endometrial cysts should improve embryonic mobility, growth during fixation, and maternal recognition of pregnancy, thereby increasing pregnancy rates. A retrospective study was performed identifying 240 mares with endometrial cyst ablations over a 10-year period. All mares had a history of being bred and not achieving a pregnancy for at least 1 cycle, or of early embryonic loss. Hysteroscopic examination was performed using a 1.68-meter long, 12.8-mm diameter fiberoptic Olympus colonoscope, on mares in diestrus or under the influence of exogenous progesterone or progestins. Uterine dilation was achieved with lactated Ringer's solution allowing both horns to be traversed and visualization of the oviductal papillae. Endometrial cyst ablation was performed using a Ceralas D25 (CeramOptec) 980 mm Nd:YAG laser fiber through the biopsy channel with penetration of the endometrial cysts at 20-22 watts in multiple regions of the cyst until lymphatic fluid was released, the epithelium heated, and collapse occurred. Mare age, number of cysts, hysteroscopic observations, postprocedure treatment, and 14 day pregnancy rates were analyzed. Statistics were performed with SAS 9.4, and all data was assessed for normality and equal variances. The impact of age on pregnancy and number of cysts was evaluated utilizing a Chi-square test. Of the 196 mares with pregnancy data, 62% of mares became pregnant following ablation of cysts. This was not impacted by age ($p = 0.958$). Most mares became pregnant in 1 (31%) or 2 (26%) estrous cycles. This was again not impacted ($p = 0.615$) by age. Age did not influence ($p = 0.276$) the number of cysts but did range immensely. Age of mares with < 2 cysts ranged from 7- 21 years with pregnancy rates 17 out of 22 (77%), 3-5 cysts ranged from 11-25 years with pregnancy rates 17 out of 19 (89%), and > 5 cysts ranged from 13-24 years with pregnancy rates 6 out of 10 (60%). The above data revealed that laser ablation of endometrial cysts improved pregnancy rates as all mares had been bred prior and failed to become or maintain a pregnancy. In addition, since the average age of the mares was 17.5 years, the pregnancy data are even more clinically relevant.

Keywords: Endometrial cysts, hysteroscopy, laser, blocked lymphatics

Effects of feeding rumen-protected choline from 21 days prepartum to 100 days postpartum on health and reproduction of Holstein dairy cows

Maria Wollentarski, Thaisa Marques, Delaine Melo, Hugo Monteiro, Rodrigo Profeta, Maria Clara Silva, Akihiro Ueda, Fabio Lima

Department of Population Health and Reproduction, University of California, Davis, CA, USA

Choline is an essential nutrient involved in lipid metabolism, methyl group donation, and cellular membrane integrity. Supplementation with rumen-protected choline (RPC) improved liver function, energy metabolism, metabolic disorders, and lactation performance. However, the impact of RPC on health and reproduction has been inconsistent. These inconsistencies have been attributed to restricted supplementation during the transition period (21 days pre and postpartum), a time-point that precedes reproductive events in dairy cows, and the nature of most nutritional studies feeding cows individually with small sample size that lacked power to identify differences in pregnancy and health disorders. Thus, we designed a study to investigate the hypothesis that feeding RPC from day 21 prepartum and extending supplementation until 100 days postpartum improves health and reproductive performance. Objective of the study was to compare the incidence of peripartum health disorders and the reproductive performance of cows fed with RPC prepartum and for an extended period of 100 days postpartum. Holstein cows were blocked by parity and randomly assigned to the control (CON; n = 389) or RPC (n = 385) group. The RPC cows were given orally 15 grams/day prepartum (–21 to 0) and 30 grams/day postpartum (0-100 days) of RPC (CholiGEM, Kemin Industries Inc.). Data were analyzed using logistic regression and Cox proportional Hazard models on JMP. Incidence of metritis did not differ ($p = 0.22$) between groups (CON = 4.9 ± 2.8% versus RPC = 3.1 ± 2.7%;). Similarly, there were no differences in milk fever (CON = 1.4 ± 1.8% versus RPC = 1.2 ± 1.8%; $p = 0.91$) and mastitis (CON = 38.0 ± 7.5% versus RPC = 37.4 ± 7.0%; $p = 0.88$). Nonetheless, the incidence of stillbirth tended to be lower in RPC than in CON cows (CON = 8.2 ± 3.5% versus RPC = 4.2 ± 3.4%; $p = 0.10$), and RPC cows had higher culling rates than CON cows (CON = 36.5 ± 6.2% versus RPC = 27.1 ± 6.1%; $p < 0.001$). The overall incidence of heel disorders tended to be lower in RPC cows (CON = 54.2 ± 3.6% versus RPC = 50.3 ± 3.3%; $p = 0.07$). For reproductive outcomes, RPC-fed cows had a delayed first estrus (CON = 34.0 ± 3.8 days versus RPC = 41.3 ± 3.7 days; $p < 0.01$) but had no difference in time to first artificial insemination (CON = 76.7 ± 2.2 days versus RPC = 77.2 ± 2.1 days; $p = 0.85$) or first service pregnancy per AI (CON = 31.5 ± 7.0%

versus RPC = 35.9 ± 6.6%; p = 0.29). However, more RPC-fed cows were pregnant by 150 days in milk (DIM) than CON herd mates (CON = 56.8 ± 7.0% versus RPC = 65.7 ± 6.8%; p = 0.03). The results of the current study underscored that extended feeding of RPF for 100 DIM led to improved tendencies in health, reduced culling, and improved reproductive outcome in Holstein dairy cows, suggesting that besides its benefits for milk yield, RPC can be a strategic supplement to support health, reproduction, and productive life.

Keywords: Rumen protected choline, pregnancy rate, stillbirth

Callicrate banding failure leading to surgical corrective castration

Taylor Yenrick,[a] Katelyn Waters,[a] Jenna Stockler,[a] Rachel Neto,[b] Carlos Lopes,[b] Thomas Passler,[a] Jessica Klabnik[a]

[a]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA
[b]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Castration via 'banding' is commonly performed by owners and requires care to ensure both testes are fully entrapped. A 10-month, 338 kg Charolais-cross steer was referred for bull-like behavior, 8 months after on farm castration by banding (Callicrate). Prior to presentation, serum submitted to Texas A&M Veterinary Medical Diagnostic Laboratory demonstrated serum testosterone concentrations of 29.10 ng/dl (< 20 ng/dl in castrated animals). On initial examination, the steer was apparently healthy with soft tissue-like structures palpable bilaterally in the inguinal regions. Ultrasonographic imaging of the inguinal structures demonstrated the presence of 2 pampiniform plexuses and surgical castration was recommended. The steer received intravenous flunixin meglumine (1.1 mg/kg once), was sedated with intravenous xylazine (0.05 mg/kg) and midazolam (0.1 mg/kg), and was placed in right lateral recumbency. An incision over the palpable tissue was made to visualize the pampiniform plexus identified ultrasonographically. Two structures resembling pampiniform plexus and testicular tissue were identified and removed. Histopathology of removed tissues confirmed them as retained, atrophied testes and associated structures with presence of spermatic cord that was obliterated in some areas followed by areas of recanalization of the arterial wall, indicating banding castration failure. Recovery was unremarkable; steer received subcutaneous florfenicol (40 mg/kg once) and Clostridium perfringens type C and D and Clostridium tetani vaccination (Barvac, Boehringer Ingelheim). This case emphasized the importance of precise placement of bands when utilized for castration. The presence of testicular and spermatic cord tissue on the right and left sides indicated either a failure to include the complete testes when placing the band or that the band had not been placed at an appropriate tightness. Appropriate usage of the Callicrate Bander and similar devices is paramount to preventing complications as described in this report.

Keywords: Castration, banding failure, surgery, cryptorchid

Large offspring syndrome in a cow

Maya Zinke, Giorgia Podico, Edgar Garrett

Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA

Large offspring syndrome is a genetic condition that can arise naturally but most commonly occurs in calves derived from in vitro fertilization (IVF) or somatic cell nuclear transfer.[1] A 7-year, Simmental cow, previously confirmed pregnant following transfer of an IVF-derived embryo, was presented for dystocia at full term. Fetal membranes were protruding from the vulva, although there were no visible indications of labor at or prior to presentation. There was a foul odor indicating the fetus had died and begun decomposing. Vaginal examination revealed minimal uterine fluid and a calf with a large edematous head. Head of the calf was removed via fetotome. After, it was discovered that the entirety of the calf was large and edematous, it was attempted to remove the right forelimb with a fetotome; however, positioning of the fetotome was difficult due to the size of the calf and lack of fluid in the uterus that resulted in inadvertent amputation of the limb at the level of the humerus. Further reduction in fetal size with the fetotome was not deemed a viable option. Cow was not a suitable candidate for a cesarian surgery due to the fetid uterine contents and an assumed reduced mobility of the uterus. This was due to the size of the calf and contraction of the uterus that prevented uterine manipulation to the level of the incision. Therefore, the cow was sedated with xylazine and euthanized with a captive bolt. Assisted reproductive techniques are commonly employed in cattle with IVF-derived embryos being the predominant technique used. This case highlighted the impact of large offspring syndrome and the necessity of research studies that help detect this condition early in pregnancy to allow early interventions.

Keywords: Cattle, dystocia, in vitro fertilization

Reference

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Citation: Clinical Theriogenology 2025, 17, 13723, http://dx.doi.org/10.58292/CT.v17.13723