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Application of domestic dog theriogenology practice in wild canids

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Abstract

Theriogenologists working in clinical practice with domestic dogs (*Canis familiaris*) have experience in managing a wide variety of subfertility cases and several aspects of breeding. Tools used include hormone monitoring, cycle manipulation, transcervical insemination, uterine and testicular biopsy, ultrasonography, and semen collection, analysis and storage. Compared to some mammalian groups, members of the family Canidae are relatively similar to each other anatomically. Species vary in their reproductive physiology and much is still unknown about how disease processes differ among species. Furthermore, susceptibility to certain diseases is not uniform throughout the clade. Diagnostic and treatment techniques developed in domestic dog theriogenology have been used with variable success in wild canids. Transcervical insemination has been used successfully in wolves and coyotes. Transcervical endometrial biopsies have been obtained and interpreted from gray wolves (*Canis lupus*), red wolves (*Canis rufus*), and African wild dogs (*Lycaon pictus*). Semen has been collected via electroejaculation and chilled or cryopreserved from numerous species. Successful use of frozen semen to obtain a pregnancy has been documented in gray wolves. Ultrasonography has been used in many species to diagnose pregnancy. Estrus induction has been used in wolves. Areas that still require much investigation include cycle monitoring and manipulation, gestational aging with ultrasonography, and semen freezing techniques.

Keywords: Canids, transcervical, cryopreservation, pyometra, ultrasonography

Introduction

Domestic dogs have been theriogenology subjects for a very long time. Some of the first artificial inseminations were on dogs in the 1700's. Breeding dogs is a widespread and active hobby and source of supplementary income for many dog owners. Because dogs have various reproductive diseases and because breeding between males and females that cannot breed naturally is desired, veterinary assistance is often sought. Clinicians who offer theriogenology services to dog breeders usually have a substantial portion of their practice devoted to these clients. Diagnostic and treatment techniques have been developed that work very well in domestic dog theriogenology practice.

Many species of wild canids are housed in zoological institutions, with some species having endangered status. Captive breeding programs aim to maintain a healthy captive population and some work with wildlife agencies to support wild populations. Whereas some species reproduce well in captivity, others are less consistently successful. Some species have a predilection for pyometra and require methods to prevent, diagnose, and treat this potentially fatal condition. Breeding recommendations from species survival plan (SSP) committees sometimes involve older individuals that have not been reproductive in years or involve individuals that are not actively in a social pair bond. Techniques used in domestic dog theriogenology practice have provided a useful framework for addressing these challenges in captive wild canids.

Breeding management

Most canids are seasonally monoestrous, with prolonged proestrus and estrus, followed by a long period of diestrus (regardless of pregnancy status), and then prolonged anestrus.[1-4] The Asian wild dog (*Cuon alpinus*) is an exception as they have a seasonally polyestrous cycle.[5] Domestic dogs are nonseasonally monestrous.[6] A common method of breeding management in domestic dogs is to monitor serum progesterone concentrations every 1 - 3 days. Progesterone increases due to preovulatory luteinization correlate with the luteinizing hormone (LH) surge, ovulation, and the fertile period.[6-7] This hormone monitoring allows accurate planning for natural breeding or artificial insemination. Breeding recommendations have developed based on changes in serum progesterone, vaginal cytology, and vaginoscopy to maximize success when using fresh, chilled, or frozen semen.[7]

Protocols are still being developed to monitor proestrus and estrus in wild canids to predict the optimal time for artificial insemination. The reproductive advisory group for the Mexican wolf SSP has tried various protocols over the past 5 years in efforts to develop an effective way to utilize frozen semen for species propagation. Logistical challenges are involved in capturing and restraining individual wolves for each blood collection, and each capture causes some degree of stress on the individual wolf. The effect of this stress on reproductive function and hormone concentrations is not known. Developing these protocols involves balancing the perceived stress of capture with the need for relatively frequent samples in order to adequately

monitor the dynamic changes during proestrus and estrus. Even with the progesterone data, it is unknown how those concentrations actually correlate with the physiological events of the LH surge, ovulation, and fertile period. Assumptions have been made that wolf profile will mirror domestic dog profile, but this has not been verified. Artificial inseminations in wolves have had mixed results, and lower success than would have been expected in domestic dogs. Over the past 5 years, many artificial inseminations with frozen semen have been performed, with only 1 verified pregnancy, resulting in a singleton birth from frozen semen at the Endangered Wolf Center in Eureka, MO in April, 2017.[8] In a study investigating evolutionary relationships between wolves and coyotes, wherein 4 female coyotes were inseminated with chilled semen from gray wolves, 2 pregnancies resulted.[9] Work is ongoing to better understand wild canid cycles and how we might better time inseminations to coordinate with their fertile periods.

Protocols have been developed to induce domestic dogs to cycle, rather than waiting for natural proestrus, by either a dopamine agonist or a gonadotropin releasing hormone (GnRH) agonist treatment. These techniques have been used successfully in wolves to induce fertile heats, as demonstrated by a number of pregnancies resulting from both natural and artificial inseminations following induced cycles. Results have been inconsistent, in part due to changing availability of GnRH products. Estrus induction can be particularly beneficial in captive breeding programs as the insemination usually involves experts from various institutions, most of whom do not live close to the institution housing the wolves. Each of these workers must take time away from their jobs and families to participate in the procedure. Waiting for the wolf to come into estrus naturally would require frequent monitoring and then everyone must come immediately when she is ready. Inducing the estrous cycle allows planning ahead of time.

Semen evaluation and preservation

Semen collection in domestic dogs is accomplished through manual stimulation of the penis. Semen evaluation techniques mirror those for other species, including basic measurements of concentration, motility, and morphology. More advanced evaluation of parameters such as DNA and membrane integrity have been established. Chilling domestic dog semen and preserving sperm function is easily accomplished now through the use of multiple commercial extenders, including but not limited to CaniPlus Chill™ (Minitube, Verona WI), BotuDog™ (Botupharma, Phoenix, AZ), Canine-EXTTM (Reproduction Provisions, Walworth, WI), and Fresh Express® (Zoetis, Parsippany, NJ). Cryopreservation of domestic dog sperm has more variable results, most likely due to individual biological variations among dogs and extender components (all of the aforementioned canine chilled semen extenders also have frozen semen extender media available).

Semen collection in wild canids is accomplished through electroejaculation under sedation. Sperm collected manually in domestic dogs maintained better motility than semen collected via electroejaculation;[10] similar effects may be present in wolf sperm, perhaps due to the prolonged exposure to prostatic fluid, which has a detrimental effect on canine sperm.[11-12] Centrifugation techniques developed in domestic dogs, using semen separators and cushions, may be of use in wild canid electroejaculated semen. Centrifugation of electroejaculated wolf semen failed to yield improvements, but had a positive effect with some extenders.[13]

Cryopreservation of wild canid semen has been practiced now for over 2 decades, but successful pregnancies using frozen semen are lacking. One litter of 6 viable gray wolf neonates and 1 stillborn was achieved following multiple vaginal inseminations with frozen semen in 1973. The cubs lived 7 days before dying from maternal neglect.[14] No other successful pregnancies from frozen semen were reported until 2017 when the previously mentioned singleton pregnancy was achieved using transcervical insemination (TCI) in a Mexican gray wolf. This overall lack of success may largely be due to the need to develop better protocols for timing inseminations. Postthaw motility is variable among males and ejaculates, but some had impressive postthaw motility percentages.

Pregnancy diagnosis and evaluation

In domestic dogs, pregnancy diagnosis may be accomplished through transabdominal palpation, relaxin assay, transabdominal ultrasonography, or radiographs. All of these require some degree of restraint. With wild canids, a relaxin assay may be the most accessible, as it only requires a blood sample, but does not give any information regarding the size or health of the litter.[15] In domestic dogs, measurements of fetal and extrafetal structures have been correlated to gestational age. Various formulas are used, depending on breed size.[16] However, these measurements have not yet been tested in wild canid species.

Subfertility evaluation

Evaluation of causes of subfertility in female domestic dogs often involves cranial vaginal cultures (using a double-guarded cultrate), transcervical endometrial biopsy[17] and culture, and ultrasonographic evaluation. Data derived from these procedures can be useful to detect inflammation, infection, fibrosis, and cystic endometrial hyperplasia.

For the male dog, subfertility evaluation involves semen evaluation, prostatic fluid evaluation, ultrasonography of the testes and prostate, and testicular biopsy. These tests can help detect infection, degeneration, hyperplasia, and neoplasia.

In wild canids, cranial vaginal and transcervical samples may also be obtained. This has been accomplished in some wild canid species, including Mexican gray wolves, red wolves, and African wild dogs. Histopathological changes appear similar among species. Both African wild dogs and red wolves are predisposed

to develop cystic endometrial hyperplasia and pyometra.[18] Early detection of cystic endometrial changes could be very helpful in management decisions.

Semen evaluation in wild canids can be done as in domestic dogs, with the exception that it is not possible to easily separate prostatic fluid from the sperm-rich fraction as the prostate continuously expresses fluid throughout the electroejaculation process. Ultrasonographic evaluations of prostate and testes, and testicular biopsy, may also be similarly performed on any wild canid, though the author is not aware of their use to date.

Conclusion

Techniques developed for use in domestic dogs can sometimes be used in their wild counterparts. Not all techniques are directly applicable, due to species differences and logistical challenges of handling wild species. Work needs to continue to investigate basic reproductive endocrinology and how to effectively manipulate cycles to more effectively manage breeding.

Conflict of interest

There are no conflicts of interest to declare.

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