

Piecing together the puzzle of carnivore reproduction

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Abstract

Recent advances in feline and canine reproductive studies demonstrate how methodically piecing this information together is beginning to reap rewards for wildlife conservation programs. Non-invasive endocrinology can be used to monitor female reproductive function, time con-specific introductions or AI, and diagnose pregnancy. Sperm morphology characteristics and cell membrane function may be genetically inherited and differ between genetically diverse and inbred species/populations in felids. It is not clear if the same is true for the endangered red wolf. While standards exist for freezing feline and canine sperm, new information using fluorescent staining and zona penetration assays (ZPA) indicates that significant damage can occur during pre-freeze cooling, and may also be related to a species' genetic diversity. Posthumous gamete salvage from genetically valuable animals not only provides a means to study sperm and oocyte physiology but also to assist with genetic management of populations. Using the knowledge gained, IVM/IVF and ICSI have been successful in the domestic cat and AI has resulted in offspring in numerous non-domestic felids. However, understanding the processes of IVM/IVF is still not well understood in canids. New information reveals that sperm and the cumulus cells may be integral to oocyte maturation and that canine epididymal sperm are not capable of undergoing fertilization. The acquisition of knowledge and application of biotechnologies lags behind for non-domestic canid conservation programs. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Scientists thirst for knowledge. It is our nature. For researchers involved with conservation programs, this thirst will only be quenched by piecing together the puzzle of endangered species' reproduction and putting this knowledge into practice to ebb the flow of species extinction. "Saving endangered species" will never be accomplished by biotechnology alone. Political landscapes, global and local economies and the drain on the world's natural resources impact too heavily to allow such simplistic solutions. Biotechnology, however, does have tremendous potential as a tool for assisting the conservation of endangered species (Wildt, 1997; Bainbridge and Jabbour, 1998; Howard, 1999), particularly with regards to small population management within zoological institutions.

Within the Order Carnivora, the Felidae and Canidae families have numerous representatives that are imperiled and could benefit from biotechnology. While an urgent need exists to develop and use assisted reproductive technologies for these species, the lack of knowledge surrounding the processes of reproduction is tremendous. Bit by bit, scientists are beginning to unravel the mysteries and put together the puzzle of reproduction for these largely elusive, heavily persecuted predators. This manuscript will attempt to highlight recent advances in feline and canine reproduction and demonstrate how methodically piecing this information together is beginning to reap rewards for wildlife conservation programs.

2. Endocrinology

Understanding endocrine patterns and inter-relationships, and how reproductive function is influenced by an individual's hormonal milieu often are overlooked in scientific studies and the development of biotechnologies. However, the basic information obtained through endocrine studies can be critical in the success or failure of species reproduction. Although resolution on a minute/hourly scale is lost, non-invasive analysis of fecal or urine hormones is effective for defining and longitudinal monitoring of reproductive events in wildlife species (Bamberg et al., 1991; Lasley and Kirkpatrick, 1991), and eliminates potential stressors associated with serum collection. Use of this technology in both retroactive and real-time analyses can augment both assisted reproductive efforts and natural breeding.

For example, the cheetah (*Acinonyx jubatus*) is notoriously difficult to breed in zoos (Marker-Kraus and Grisham, 1993). Females often do not display overt estrous behavior, and recent data have demonstrated that multiple females maintained at the same institution can experience random periods of anestrus throughout the year, perhaps due to cage-mate suppression (Brown et al., 1996). Fecal steroid analysis can demonstrate whether or not a female is experiencing estrous cyclicity (based on regular episodic fluctuations in estrogen content), and help time male–female introductions. If behavioral estrus or copulation is not observed, analysis of fecal progestins can confirm mating/ovulation and subsequently diagnose a pregnancy or pseudopregnancy (Fig. 1).

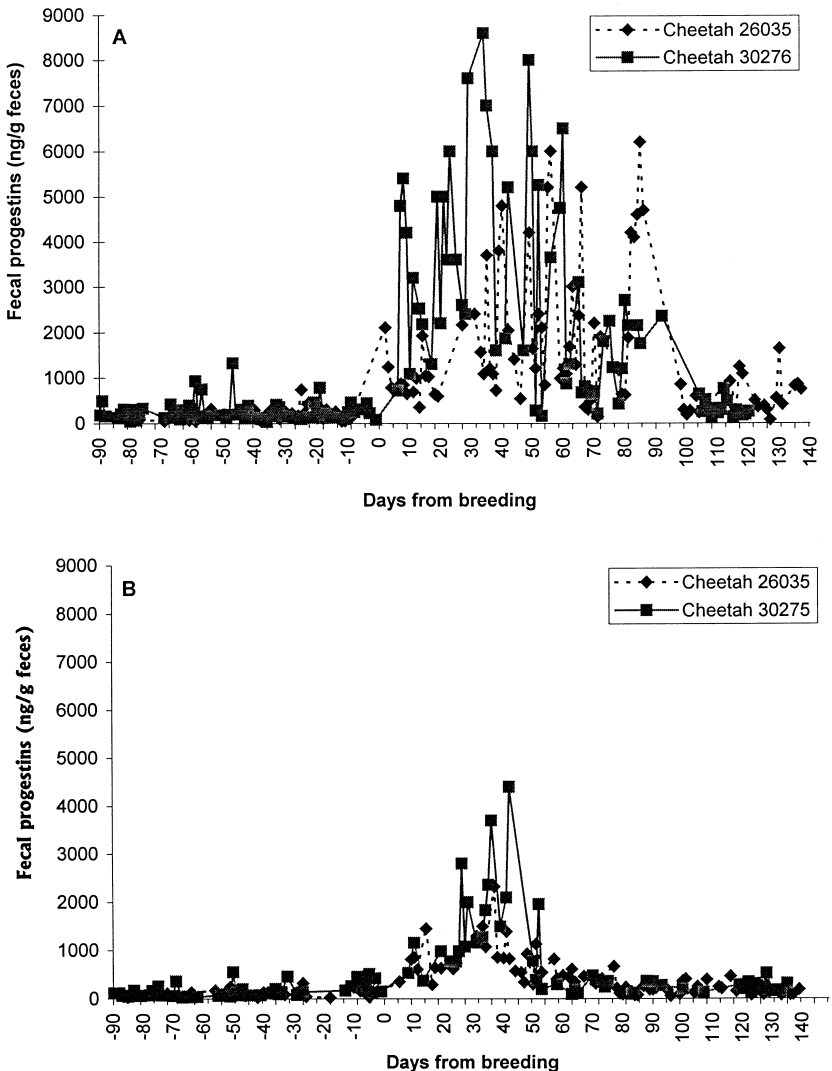


Fig. 1. Fecal progesterin profiles from two pregnant (A) and pseudopregnant (B) cheetahs demonstrating differences in hormonal concentrations and duration of progesterin secretion. Data are aligned to the first day of observed or presumed breeding (Day 0). For both reproductive states, fecal progesterins begin a sustained rise 2–4 days after breeding. Progesterin values were greater and were sustained for longer duration in pregnant vs. pseudopregnant females.

This approach is more precise and less labor intensive than random male–female introductions to achieve breeding. Both Brown et al. (1996) and unpublished data from our laboratory have demonstrated an approximate 67-day pseudopregnancy (range 53–78 days, two-thirds of gestation length) with a precipitous drop in progesterin values,

compared to a 92–94-day gestation, in which progesterins remain elevated approximately 10-fold above baseline until near the time of parturition. We also have used this approach to diagnose ovulation and pregnancy in a lioness (*Panthera leo*). During a period of estrus, breeding was observed, but ovulation did not occur (Fig. 2A), perhaps due to an insufficient number of copulatory stimuli. During the following estrus, copulation occurred and was followed by ovulation (Fig. 2A); pregnancy subsequently was diagnosed (Fig. 2B, 1998 data)

Regardless of species, significant between animal variation in fecal steroid levels results in a need to examine longitudinal profiles on an individual basis to ensure accurate diagnoses. Although this approach may not be classified as “assisted reproduction,” determining if a female is experiencing estrous cyclicity, the appropriate time to

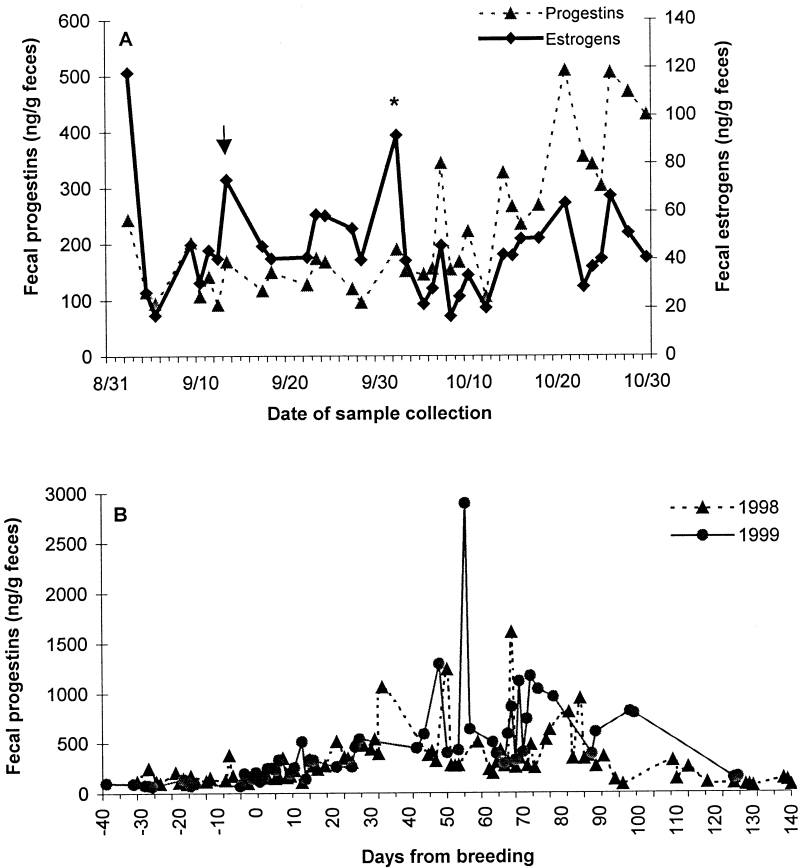


Fig. 2. Fecal hormone profiles from a single female lion. A rise in fecal estrogens on 9/13 (arrow) was associated with a single copulation (A), but ovulation did not occur based on the lack of a progesterin rise. A subsequent series of copulations observed on 10/2–4 (*) was associated with elevated estrogens. Based on a sustained rise in fecal progesterins, ovulation was presumed to occur. Fecal progesterin profiles (B) associated with two pregnancies in the same female. Data are aligned to the first day of observed copulation (Day 0).

introduce her to a male, confirmation of ovulation and diagnosis of pregnancy does assist reproduction. While not as high profile as producing cubs by artificial methods, natural reproduction generally is preferred.

We have used this approach in red wolves (*Canis rufus*) during three previous breeding seasons (February–March, 1996–1998) to characterize three female reproductive classes: (1) cyclic, non-pregnant; (2) cyclic, pregnant and (3) acyclic (i.e. non-ovulatory). The acyclic class was identified by elevated levels of fecal estrogens and progestins for up to 5 weeks prior to the anticipated time of estrous, thereby providing a predictor of infertility on an individual basis (Walker, 1999). This not only can assist species management for the current breeding season, but also provides useful information when making decisions on future breeding pairs. Similar endocrine methods have been validated for the maned wolf (*Chrysocyon brachyurus*; Wasser et al., 1995) and African wild dog (*Lycaon pictus*; Monfort et al., 1997), but still remain to be implemented on a practical basis.

In felids, follicular development and ovulation can be induced through gonadotropin regimens, but with significant inter-species variation (Brown et al, 1996; Howard et al., 1997a). This is not the case with canids, in which the estrous cycle cannot be routinely controlled through the administration of exogenous hormones. The general lack of understanding for mechanisms controlling the canine estrous cycle leaves a large gap towards developing assisted reproductive techniques in canids. In addition, many non-domestic canids are seasonally monoestrous; therefore, detection of ovulation for timing breeding or artificial insemination becomes a critical piece in the puzzle of reproduction. Although canine estrous cycles can be monitored through fecal hormone analysis and fecal hormones do mimic serum profiles in the peri-ovulatory period (Gudermuth et al., 1998; Hay et al., 2000), due to significant between animal variation, there is no “magic” or specific progestin value (as in serum) to use as a predictor for AI. Instead, investigators must longitudinally monitor each female to detect an estrogen peak followed by a subsequent prolonged progestin elevation. Based on the correlation between fecal and serum profiles, we estimate that AI with fresh semen should be conducted approximately 4–6 days after the fecal progestin rise (defined as an elevation that is 2 standard deviations above an individual’s baseline value and which is maintained for at least 3 days; Walker (1999)).

Due to the nature of canid pseudopregnancy, it generally is accepted that pregnancy cannot be diagnosed through serum steroids. Gudermuth et al. (1998) have demonstrated that pregnancy diagnosis is possible through fecal hormone analysis, as they found elevated levels of fecal progestins, estrogens and testosterone in pregnant compared to pseudopregnant bitches. The authors attribute differences in results between fecal and serum analyses to increased hemodilution, metabolism and serum clearance rates in pregnant females. We have observed similar trends in the red wolf (Walker, 1999), but cannot make statistical inferences between pregnant and pseudopregnant females.

Endocrine studies also can impart knowledge regarding the well being of an animal. It is well known that chronic or acute stress can negatively impact on reproductive function (see review, Liptrap (1993)). To accurately monitor “stress,” samples must be collected without interfering in an animal’s routine to define normative profiles. In felids, this has been accomplished through measurement of urinary corticosteroids

(Carlstead et al., 1992, 1993). After establishing baseline values for adrenal function in domestic cats and three non-domestic felid species, animals were exposed to novel or stressful environments. In all cases, significant changes from baseline urinary corticosteroids were observed.

Preliminary evidence suggests that a similar approach may be effective for canids. Following validation of a fecal cortisol assay for red wolves (Walker, 1999), corticosteroid levels were longitudinally evaluated in two female red wolves: the first was naturally bred, and the second was restrained on a frequent basis to obtain serum for progesterone analysis. In the naturally bred female, fecal corticosteroid values remained at baseline (Fig. 3A); whereas in the second female, repeated restraint episodes were associated with elevated corticosteroids (Fig. 3B). While neither the felid nor canid examples demonstrate that elevated adrenal responsiveness negatively impacts on reproductive function, they do illustrate that environmental factors or repeated restraint episodes may result in altered corticosteroids and that the methodology exists to further study the effects of stress on reproductive function in carnivores.

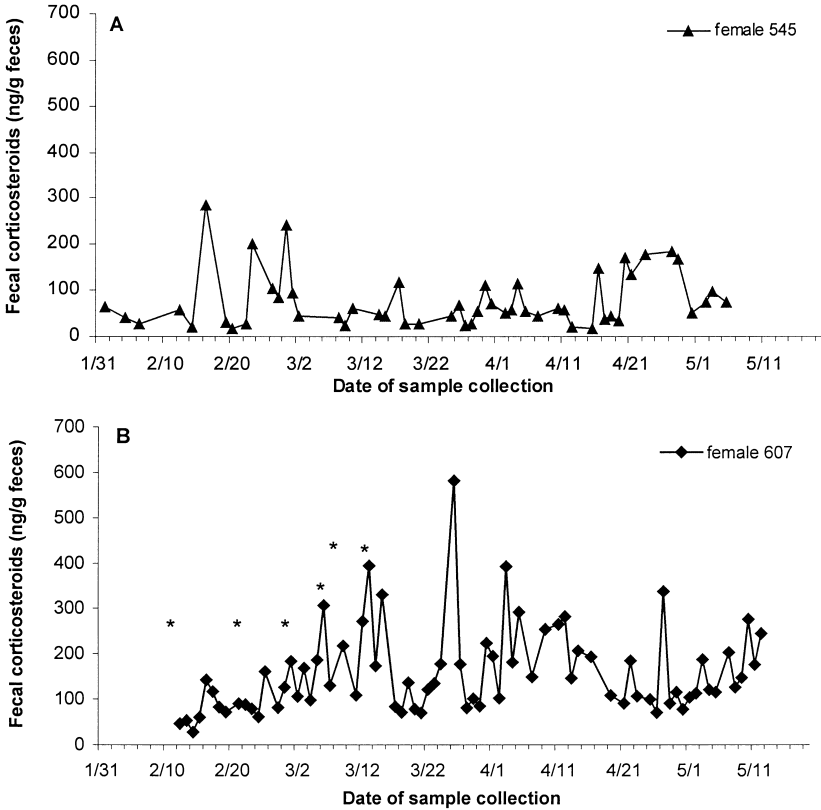


Fig. 3. Fecal corticosteroid profiles from a naturally bred (A) and repeatedly restrained (B) red wolf. Asterisks indicate restraint episodes. The onset of estrus was between 3/1 and 3/12 for both animals.

3. Factors influencing fertilization and embryo development are not just in the culture dish

Fertilization can be affected by variables in both the sperm and the oocyte. Examination of sperm function in domestic and non-domestic cats has been conducted using intra- and inter-species zona penetration assays (ZPA) with both fresh and salt-stored zonae. Domestic cats have been classified into two groups based on the percentage of morphologically normal sperm present (normospermic, > 60% normal; teratospermic, < 40% normal), with ejaculates from these two groups portraying different gamete interaction qualities (Howard et al., 1993). The fertilization rate using sperm after swim-up was 94.6% vs. 53.2%, and penetration of salt-stored zonae was 73.7% vs. 24.1% for normospermic and teratospermic individuals, respectively. The mean number of bound sperm/ovum also was reduced from 26.2 for normospermic individuals to 8.3 for teratospermic individuals (Howard et al., 1993). Low zona penetration and/or reduced IVF rates also have been observed in felid species traditionally characterized by large proportions of abnormal sperm (cheetah, Donoghue et al. (1992); clouded leopard, *Neofelis nebulosa*; Long et al. (1996a)). The reason for this reduced sperm function appears to be the greater degree of ultrastructural acrosome abnormalities observed in teratospermic cats (Long et al., 1996b).

Further investigation has identified two sperm-membrane associated tyrosine phosphorylated proteins in cats. A decrease in phosphorylation of these proteins is associated with compromised capacitation and acrosome reaction in teratospermic cats (Pukazhenthii et al., 1996). These proteins now have been shown to be present in four non-domestic felid species: two traditionally characterized as normospermic (tiger, *Panthera tigris*; leopard cat, *Felis bengalensis*) and two generally characterized as teratospermic (cheetah, clouded leopard). Similar to the domestic cat, the teratospermic species demonstrated attenuated tyrosine phosphorylation compared to the normospermic species (Pukazhenthii et al., 1998). The conclusion made from these progressively stepwise studies is that diminished protein tyrosine phosphorylation is likely one factor responsible for the reduced sperm function observed in teratospermic individuals/species.

In felids, low genetic variability within species populations has been associated with poor ejaculate characteristics in cheetahs (Wildt et al., 1983; O'Brien et al., 1985), lions (Wildt et al., 1987), pumas (*Felis concolor*; Roelke et al. (1993); Barone et al. (1994)). The underlying reasons for these poor ejaculates have been investigated in two distinct populations of African lions: (1) an outbred group from the Serengeti plains and (2) a group geographically limited to the Ngorongoro Crater, in which restricted migration has led to inbreeding (Wildt et al., 1987). While there do not appear to be differences in hypothalamic, pituitary and testicular endocrine function (Brown et al., 1991), the numbers of seminiferous tubules, spermatids and interstitial areas are different between the two groups (Munson et al., 1996). Tied together with the poor fertilizing ability and reduced protein phosphorylation described above, it is speculated that there is an inherent compromised sperm function related to low genetic variability and that the effects of inbreeding are manifested at the gonadal level during development and are later reflected in spermiogenesis.

In contrast, although dog semen has been well characterized, populations of “normospermic” and “teratospermic” animals or even groups of fertile and infertile animals have not been routinely or methodically examined. Nevertheless, homologous zona (Hay et al., 1997b; Hewitt and England, 1997) and hemi-zona (Mayenco-Aquirre and Perez Cortes, 1998) penetration assays have been established for the domestic dog. The ZPA has been used to differentiate sperm binding abilities in small numbers of fertile ($n = 7$) and infertile dogs ($n = 3$; Mayenco-Aquirre and Perez Cortes), compare sperm capacitation techniques (Hewitt and England, 1997) and evaluate damage to sperm caused by cooling and freezing (Hay et al., 1997a,b).

Due to the nature of zoological collections, large numbers of species representatives usually are not maintained at one location. Therefore, for these assays to be useful for non-domestic canid semen evaluation, the system must be portable and zonae readily accessible where the canids of interest are located. In our laboratory, the domestic dog is used as a model for the red wolf to investigate factors that impact on the success of portable research systems.

Unfortunately, we have discovered that unlike felids, salt storage is ineffective for the canine ZPA, as evidenced by attenuated sperm penetration (Hay et al., 1997b), leading us to explore other alternatives for obtaining gametes in remote locations. Using oocytes that remained intact within the ovary and were stored for either one night (97.0% penetration, 13.8 sperm/ova, $n = 238$ oocytes, seven ejaculates) or two nights (92.4% penetration, 12.6 sperm/ova, $n = 103$ ova, three ejaculates) in a refrigerator in phosphate buffered saline provided equivalent zona penetration results (Mastromonaco and Goodrowe, unpublished data) compared to freshly collected oocytes (94.9% penetration, 11.7 sperm/ova). Alternatively, culturing oocytes for 24 h prior to the ZPA provided similar numbers of sperm/ova (5.6), but resulted in a reduced percentage of zonae penetrated (56.5%) compared to control values (6.9% and 80.9%, respectively, $n = 5$ ejaculates; Ryckman and Goodrowe, unpublished data). Therefore, only fresh canine oocytes or ovaries cooled 24–48 h may be used to assess sperm function with the ZPA.

To render the ZPA transportable, we have tested a portable incubator system consisting of a water- and airtight plexiglass chamber filled with $\sim 5\%$ CO_2 and submerged in a 38°C water bath. The source of CO_2 for the portable incubator can be either exhaled air or 5% CO_2 in air from a tank. In both cases, ZPA results (exhaled air: 90.8% penetration, 7.8 sperm/ova; CO_2 tank: 76.1% penetration, 9.0 sperm/ova) were equivalent compared to control values in a standard incubator environment (90.3% penetration, 9.8 sperm/ova; Ryckman and Goodrowe, unpublished data)

Because of the small number of founder animals for the present red wolf population, it has been suggested that reduced genetic diversity may negatively impact on reproduction in this species (Koehler et al., 1994, 1998). Currently, there is no direct evidence that inbreeding has affected the red wolf, since fecundity remains high and inbreeding coefficients low. However, we have found that red wolf sperm quality is somewhat reduced compared to domestic dogs and grey wolves. In particular, red wolf sperm demonstrate a high proportion of bent/coiled tails (Koehler et al., 1998, Goodrowe et al., 1998), abnormally shaped sperm heads (7.3%) and partial or missing acrosomes (35.2%, $n = 10$ ejaculates; Goodrowe et al., unpublished data, 1999; Koehler et al., 1998). Collectively, the first assumption was that sperm characteristics for this species

were poor. However, we have found that the osmolarity of red wolf seminal plasma (279.1 mOs; $n = 11$) varies greatly between individuals (range: 204.5–340 mOs) and is below that of domestic dog seminal fluid (303.9; $n = 4$) and typical culture solutions. Therefore, processing red wolf sperm in a manner similar to that routinely used with domestic dogs may exacerbate the high degree of morphologically abnormal cells. Ongoing studies in this species to test different processing methods in conjunction with examination of relationships between ejaculate characteristics and inbreeding coefficients may shed light on whether attenuated sperm characteristics are genetically inherited or induced by *in vitro* treatment.

4. Semen freezing process

Within the felid family, the standard protocol for sperm cell freezing involves a 30-min cooling period in an egg-yolk-based extender in a 5°C environment. Cooled semen is pelleted onto dry ice, allowed to freeze for 3 min and then plunged into liquid nitrogen. (Howard, 1986). Freeze-thawing with this method has resulted in pregnancies in domestic and non-domestic felids (Howard, 1999), but inconsistencies between sperm characteristics and AI success have led investigators to examine cellular damage during cooling. The above protocol ($\sim 4^\circ\text{C}/\text{min}$) induces substantial acrosomal membrane damage, particularly between 25–17°C, without changing motility (Pukazhenthil et al., 1999). When cooling rates were slowed to 0.5°C/min, the percentage of intact acrosomes was improved by almost 20% and 40% for normospermic and teratospermic cats, respectively.

Semen cryopreservation methods for dogs and foxes have been well-reviewed by Farstad (1996). Although semen cooling and freezing is commonplace for these two species, work with endangered wolf species has not fared as well. Our recent studies with red wolves suggest that cooling is a major source of cell damage. In 1999, semen was collected from 10 male red wolves by electroejaculation, resulting in 75.7% normally shaped sperm, with 72.9% and 70.4% normal acrosomes using previously described Spermac and PSA/FITC staining methods, respectively (Hay et al., 1997b; Goodrowe et al., 1998). After cooling six ejaculates for 30 min in a 5°C refrigerated environment ($\sim 0.6^\circ\text{C}/\text{min}$) using previously described methods (Goodrowe et al., 1998), which have been proven successful for domestic dogs (C. Platz, personal communication), normal morphology (75.8%) and the proportion of normal acrosomes observed with Spermac staining (77.7%) remained similar to pre-cool samples. However, examination by PSA/FITC staining demonstrated only 22.7% normal acrosomes. These results were mimicked when sperm cells from four ejaculates were cooled in a water bath at a rate of 1.1°C/min, using a previously described method for the domestic dog (Hay et al., 1997b). Normal morphology was slightly reduced to 59.7%, with 65.2% normal acrosomes as evidenced by Spermac staining. Using PSA/FITC staining, the percentage of normal acrosomes was slightly improved to 35.7%. Collectively, these data suggest that a major portion of cellular damage in red wolf sperm occurs during the cooling process, long before freezing takes place. Current methods for dog semen cryopreservation do not appear to be optimal for red wolf sperm, and new cooling

methods are required for this non-domestic canid. Reasons for differences in membrane susceptibility to cooling damage remain to be clarified, but may be an indicator of general reduced sperm function in the red wolf, as is observed with teratospermic domestic and non-domestic felids.

5. Is posthumous reproduction possible?

Gamete salvage from genetically valuable animals, particularly those which die suddenly or unexpectedly, is a potentially powerful tool for conservation programs as a means of maintaining population genetic diversity. Although posthumous gamete recovery is frequently used in farm animal species for studies of gamete biology, and in some instances offspring production, these practices are not presently standard for carnivores.

In vitro oocyte maturation and subsequent fertilization has been shown to be relatively effective in the domestic cat and several non-domestic feline species using relatively standard culture conditions supplemented with combinations of FSH, LH, estradiol and BSA (Johnston et al., 1989, 1991; Lengwinat et al., 1992). Maturation to MII is in the range of 60%, with fertilization rates ranging from 40–60%. Selective separation of oocytes based on the appearance of the cumulus cell complex results in more consistent maturation and fertilization rates and provides an indicator of oocyte maturation and developmental ability (Wood and Wildt, 1997). Use of feline oviductal cells also improves the incidence of zygotes (49.5% vs. 34.9%) and eight-cell embryos (48.6% vs. 30.3%) using epididymal sperm for fertilization (Lengwinat et al., 1992).

Interestingly, felid ovaries can be stored up to 24 h at 5°C, with IVM/IVF and blastocyst development rates (~60% MII, 9% blastocysts) comparable to freshly collected oocytes (~60% MII, 13% blastocysts; Wolfe and Wildt, 1996). While oocytes from ovaries stored at 5°C for 48 h demonstrated 25% cleavage after IVM/IVF, blastocyst development was not observed, indicating that prolonged cold storage is detrimental to the developmental capacity of feline oocytes.

This information has tremendous implications for conservation programs. Laboratories with the appropriate equipment and expertise to carry out IVM/IVF of feline oocytes are few in number and are geographically dispersed. Cold storage of ovaries in conjunction with overnight shipment would enable production of embryos from a genetically valuable animal, thus completing a critical step towards using biotechnology for posthumous reproduction.

In contrast to felids, the collective knowledge of canine oocyte biology and maturation is poor. Studies over the past few years (Bolamba et al., 1998; Durrant et al., 1998; Hewitt and England, 1998; Hewitt et al., 1998) have examined the feasibility of IVM, but maturation rates to the MI/MII stage still remain low (<20%). The few existing pieces of evidence suggest that oocytes originating from dogs of breeding age (7–48 months, Bolamba et al., 1998; Durrant et al., 1998; 1–6 years, Hewitt and England, 1998) exhibit greater maturation rates than older dogs, and that higher concentrations of protein (BSA or fetal calf serum) result in greater maturation rates (Hewitt et al., 1998). One fascinating discovery made in a preliminary study is that the presence of canine sperm in an oocyte culture environment enhances the incidence of condensed chromatin

from 9–42% (Hay et al., 1994), suggesting that canine sperm may play an integral role in dog oocyte maturation. This hypothesis is strengthened by our recent observations in red wolf ZPA studies, where 11% of dog oocytes (12/109) demonstrated maturation beyond the GVBD stage when cultured with red wolf sperm overnight in the portable incubator system (unpublished results). While the stage of oocyte maturation does not appear to impact on sperm binding (Hewitt and England, 1997), cumulus cells do appear to be critical this event. Compared to control values, a marked reduction in the mean number of sperm/zona (11.7% vs. 4.1) and the percent of zonae penetrated (94.9% vs. 55.1%) has been observed when cumulus cells were removed prior to co-culture of sperm and oocytes (Mastromonaco and Goodrowe, unpublished result) for the ZPA.

The differences between the feline and canine families regarding the possibilities for gamete salvage are not limited to oocytes, but also extend to sperm cells. Feline epididymal sperm readily penetrate oocytes in vitro (Goodrowe and Hay, 1993) and are capable of fertilizing oocytes in vitro either fresh (40.7% cleavage) or after freeze-thaw (25.3%; Lengwinat and Blottner, 1994). In contrast, canine epididymal sperm penetrate homologous zonae only at very low rates in comparison to ejaculated sperm (36.1% vs. 73% zona penetration; 1.7 vs. 4.1 sperm/ova; Bateman et al., 2000; Hay et al., 1997b). Acrosomes from epididymal sperm also demonstrate a substantial increase in partial acrosomes and decrease in intact compared to ejaculated sperm (Bateman et al., 2000). We speculate that this may be due to incomplete acrosomal membrane contents and/or maturation. Collectively, these data suggest that canine epididymal sperm are not capable of routinely undergoing fertilization, and therefore would not be effective for posthumous fertilization without cell modification.

6. Putting the pieces together

Assisted reproductive technologies have by far been more successful in conservation programs for non-domestic felids vs. canids. This is surprising, given the active commercial industry for domestic dogs. The most recent and significant advances in carnivore biotechnology have been the birth of kittens by IVM/IVF and ICSI (Pope et al., 1997, 1998) in the domestic cat, demonstrating the feasibility of gamete salvage within this family. Additionally, kittens/cubs in a variety of large and small non-domestic felids (puma, cheetah, tiger, clouded leopard, leopard cat, ocelot, *Felis pardalis*; snow leopard, *Panthera uncia*) have been born as a result of artificial insemination (see review, Howard (1999)).

While observations in the domestic cat indicate that treatment with exogenous gonadotropins followed by artificial insemination does not impair an individual's ability to produce good quality embryos (Roth et al., 1997), dosages and timing of administration must be established for each species (Howard et al., 1997a, Howard, 1999). Use of endocrinology to complement studies of ovarian responsiveness to exogenous hormonal therapy is warranted to gain further insight into species-specific mechanisms of gonadotropin responses.

There presently is one instance that demonstrates the tremendous potential which assisted reproductive technology can have for conservation programs. To meet the

necessity of providing new founder lines to captive populations while at the same time avoiding animal removal from the wild, Howard et al. (1997b) recently collected and froze semen from a male cheetah in Africa. The frozen sample was translocated to the Albuquerque Zoo and used to inseminate a resident female. This resulted in the birth of a single cub, demonstrating that genetic information can be collected from free-ranging animals and used to supplement captive breeding populations.

Although success in non-domestic canids has been less frequent, it is only recently that repeated efforts to perform AI have been made. One litter of wolf pups was reported following AI (Seager et al., 1975). Using serum progesterone to time insemination, a single pregnancy in the red wolf has been accomplished following surgical intrauterine semen deposition (Waddell and Platz, personal communication, 1995).

Computer-assisted population modeling has demonstrated that artificial insemination with either fresh or frozen-thawed sperm likely will be sufficient to meet genetic management goals or improve genetic diversity in small populations of carnivores (Wildt and Seal, 1994). Therefore, except in extreme cases, use of techniques such as IVM/IVF, ICSI and cloning likely will not be warranted for carnivores. The exceptional amount of time and financial resources needed to perfect these techniques on a species by species basis likely will serve as a deterrent to develop such sophisticated technology for endangered species. From a genetic population management perspective, cloning generally is not a desired path, as the endpoint is genetically identical individuals and zoos strive to achieve populations with maximum genetic diversity, with low inbreeding coefficients. Nevertheless, these technologies offer extremely important tools for studying gamete biology and interaction and offer novel means to provide more links in the puzzle of carnivore reproduction.

7. Conclusions

Over the last decade, the emphasis in feline and canine reproduction has been primarily aimed at solving the puzzles associated with reproductive mechanisms. By using methodical approaches, scientists have been able to fit critical pieces of information together, resulting in significant practical applications of biotechnology for domestic and non-domestic species. Nevertheless, many gaps still remain in the puzzle of carnivore reproduction, particularly with reference to the impact of genetic diversity on reproductive function, leaving scientists still parched in our thirst for knowledge and desire to impact on wildlife conservation programs.

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