DEVELOPING ASSISTED REPRODUCTIVE TECHNOLOGIES TO PROMOTE *EX SITU* RAPTOR CONSERVATION

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Of the 307 species in the Order *Falconiformes* (diurnal birds of prey), 31 (10%) are listed as globally threatened. Substantial progress has been made during the past 4 decades in managing *ex situ* populations. However, apart from the excellent results obtained with selected falcons (e.g., Peregrine falcon) and vultures (e.g., California condor), consistent captive breeding remains elusive, especailly for endangered eagles and hawks. This is largely due to unknown biological norms for the various species (Donoghue et al., 2002) as well as a likely susceptibility to captivity stress. Little information is available for virtually all birds of prey. Additional challenges with raptors include the unavailability of founders, inbreeding depression, female-male incompatibility (i.e., aggression), asynchrony, disability for natural copulation, poor seminal quality, urine contamination, sperm transport inefficiency and disease. The objective of our studies has been to use a multidisciplinary approach (Wildt et al., 2001) to first understand the basic reproductive biology of selected raptors and then to apply this new knowledge to developing assisted breeding. Five raptor species have been studied as 'models' or due to their level of endangerment: Spanish Imperial eagle (*Aquila adalberti*), Golden eagle (*Aquila chrisaetos*), Bonelli´s eagle (*Hieraaetus fasciatus*), Booted eagle (*Hieraaetus pennatus*) and Peregrine falcon (*Falco peregrinus*).

Using the massage semen collection technique, there was a high incidence of urine contamination (mean ± SEM): Spanish Imperial eagle, 43.1 ± 9.1% of all ejaculates; Golden eagle, 36.8 ± 12.8%; Bonelli's eagle, 28.7 ± 16.1%; and Peregrine falcon, 48.2 ± 17.3%. Urine acidified the ejaculate, which caused marked decreases in sperm motility and viability. This problem was circumvented by immediate, gentle seminal washing with modified Lake´s diluent (Blanco et al., 2002).

Approximately 10% of Spanish Imperial eagles produced teratospermic ejaculates in which 84.6 ± 12.2% of sperm were pleiomorphic. Of 5,950 ejaculates from the five species, 4,998 (84%) were judged to be suboptimal or poor quality. This finding combined with subjective observations of female 'stress' during artificial insemination (AI) and naturally poor transport of vaginally deposited sperm likely explain the lack of assisted breeding success. One novel approach has been an intramagnal insemination method (Blanco et al., 2002) that has allowed the production of fertile eggs, even when low quality inseminates are used. In a preliminary trial, two of 12 (Peregrine falcon) and one of nine (Golden eagle) eggs hatched using washed sperm and this AI technique. Parallel endocrine evaluations of female excretory patterns of estrogen and progesterone

prior to egg laying have been useful for predicting time to first ovulation while identifying optimal time for AI. We expect that this multidisciplinary approach will maximize fertility while minimizing numbers of sperm required per AI.

Extensive comparative studies also have been conducted to evaluate sperm tolerance to cryoprotectant and temperature. Raptor sperm differed remarkably in response to changes in the surrounding medium during freezing-thawing. Raptor sperm viability at osmolarities ≥800 mOsm was higher ($P < 0.05$) than in similarly treated poultry (i.e., chicken and turkey) sperm (Blanco et al., 2000). Moreover, significant differences were found between families (*Accipitridae* and *Falconidae*), and also among eagles within the same family. For example, compared to the Bonelli's and Booted eagle, sperm from the Imperial eagle were most resistant to changes in osmolarity. Increasing concentrations of dimethylacetamide, a permeating cryoprotectant, decreased ($P < 0.05$) the number of surviving sperm in all the species. Sperm membrane damage in the presence of cryoprotectant varied among species and with equilibration temperature. Maintaining semen at refrigeration (4ºC) temperature was beneficial for sperm from all but the Bonelli´s eagle; however, sperm damage in this species decreased when dimethylacetamide was added at room (21ºC). Compared to slow cooling (1ºC/min), rapidly cooling semen (50ºC/min) decreased ($P < 0.05$) sperm motility in all except the Spanish Imperial eagle which tolerated even fast cooling. A clearer understanding of this species uniqueness may provide clues to developing sperm freeze-thaw techniques for other raptors. Recent observations also revealed individual male difference in sperm tolerance to cold stress. For example, although Peregrine falcon sperm generally did not survive rapid cooling, two of 23 males produced ejaculates that resisted cold-induced damage. A comparative analysis of membrane composition post-thawing indicated a marked increase ($P < 0.05$) in cholesterol and lipid domain integrity in the sperm of males most tolerant to freeze-thawing.

Studies also demonstrated that most acrosome damage occurred from –23º to –196ºC and during subsequent re-warming. Moreover, rapid cooling exerted its damage from +4ºC to –23ºC in all species, but again the acrosome of Spanish Imperial eagle sperm was most resistant to cryodamage compared to species counterparts ($P < 0.05$). For example, only 78.3 ± 4.3% of the acrosomes of Peregrine falcon sperm remained intact during the temperature decline from +4ºC to –23ºC. Unlike in all eagle species, increasing concentrations of cryoprotectant failed to improve acrosomal integrity in the falcon. These differences likely are due to species specificities in acrosomal morphology and composition.

Finally, there was a high concentration of spermatogonia in Peregrine falcon semen (268 ± 10.4 cells/μl) compared to the Spanish Imperial (20 ± 4.2), Bonellí's (15.2 ± 6.6) or Golden (17.6 ± 4.6) eagle ($P < 0.05$). This finding has motivated explorations into cryopreserving spermatogonia. Interestingly, Peregrine falcon spermatogonia preferred slow cooling (0.25ºC/min), whereas falcon sperm preferred a moderate cooling rate (1ºC/min). Additionally, optimal post-thaw survival for falcon spermatogonia occurred in the presence of dimethylacetamide, whereas dimethylsulfoxide was better for freeze-thawing falcon sperm.

We conclude that there is significant specificity in reproductive biology norms among birds of prey, even for species that are taxonomically related. Although there is much room for improving the quality of semen that can be collected from raptors, some males consistently produce good-to-excellent quality semen. However, there are remarkable differences among species in processing needs to ensure viability *in vitro* or to withstand cryopreservation. The key to success is detailed and systematic basic research in both physiology and endocrinology. When sufficient information is generated, it is possible to produce offspring by AI. We predict that such methods will contribute in the future to better genetic management of rare *ex situ* populations.

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