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Semen collection and characterization of normative reproductive traits in free-ranging ocelots (*Leopardus pardalis*) and bobcats (*Lynx rufus*) in southern Texas

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ABSTRACT

Decreased genetic diversity and possible inbreeding depression have recently been documented in the last wild ocelot (*Leopardus pardalis*) population in the United States. One consequence of inbreeding depression in felids may be reduced semen quality which can adversely affect reproductive potential. Detailed assessments of reproductive parameters in wild individuals and populations can be conducted using assisted reproductive technologies, such as semen collection and analysis. For most felid species, semen has traditionally been collected via electroejaculation (EEJ[2]); however, an alternative method has been developed using alpha-2 agonist drugs to induce direct sperm release into the urethra, allowing collection by catheterization without requiring specialized equipment. The goal of this study was to characterize normative reproductive traits in free-ranging ocelots and co-occurring bobcats (*Lynx rufus*) in southern Texas and assess the effectiveness of urethral catheterization (UC[3]) for semen recovery in both species. For semen collection, free-ranging cats were live-captured and anesthetized using intramuscular ketamine and medetomidine/dexmedetomidine (alpha-2 agonist) with UC conducted 20–40 minutes post-induction. In ocelots only, EEJ was subsequently performed if UC failed to recover a viable sample. Semen collection was attempted in 31 felids (n=9 ocelots; n=22 bobcats), with sperm recovery by UC in seven of nine ocelots (78 %) and 14 of 22 bobcats (66 %), and by EEJ in four of five ocelots (80 %). For ocelots, the percentage of primary morphologic abnormalities was higher ($p<0.001$) for UC (47.75 ± 6.7; mean ± SEM) compared to EEJ (9 ± 2.7) but percent normal morphology (MORPH) did not differ between UC and EEJ ($p=0.218$). In wild ocelots, seminal parameters appeared lower relative to historical values reported for zoo-managed ocelots, possibly related to reduced heterozygosity. In wild bobcats, seminal traits were inferior to those of ocelots but similar to reports for other zoo-managed *Lynx* species. In conclusion, detailed male reproductive traits have been characterized for the first time in wild, free-ranging ocelots and bobcats in southern Texas. Although UC allowed semen recovery for assessment of seminal traits in both species, EEJ produced higher quality samples in ocelots when applied after UC while also mitigating the adverse impact of urine contamination observed frequently with both collection methods.

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[3] Urethral Catheterization

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1. Introduction

Several wild felid populations have been found to exhibit low levels of genetic heterozygosity, including in southern Texas, where evidence of decreased genetic diversity and inbreeding were recently documented in ocelot (*Leopardus pardalis*) populations [1–5]. Genetic drift and inbreeding can compromise fitness and interact with demographic stochasticity to further reduce population viability [6–8]. The small ocelot population in Texas (estimated to be < 80 individuals), coupled with its isolation and fragmentation, has enabled genetic drift and inbreeding, leading to lower genetic diversity when compared to larger connected ocelot populations [2]. Although bobcats (*Lynx rufus*) in southern Texas have greater genetic diversity than ocelot populations found in the same region, bobcats living along the Lower Rio Grande Valley have shown relative declines in genetic variation [9]. Bobcats occur throughout the US, extending from southern Canada to northern Mexico [10], and are currently listed by the IUCN[4] as a species of least concern and a stable global population [11]. Bobcat populations in some regions, however, may be adversely affected by loss of habitat, as well as disease and intensive trapping, raising some concerns about the future sustainability of some populations throughout their range.

One possible consequence of inbreeding in wild felids is the reduction of semen quality, ultimately decreasing breeding success and accelerating population decline. Reproductive examinations and semen analysis allow detailed descriptions of male reproductive traits to assess the possible impact of inbreeding depression on various aspects of seminal quality and viability. Basal reproductive traits typically characterized in male felids include testicular volume, ejaculate volume, sperm concentration, percent motility and rate of forward progression, penile and sperm morphology, acrosome status, and serum and fecal testosterone concentrations. In other wild felid populations exhibiting reduced genetic variation, a variety of compromised seminal qualities have been observed including increased percentages of structurally defective spermatozoa [4,12–14], reduced sperm concentration [14], decreased total motile sperm numbers [5] and higher percentages of acrosomal defects that render sperm deficient in fertilization potential [5,15,16]. This decrease in semen quality may impair successful conception, ultimately limiting further population growth and leading to extinction.

Although assisted reproductive technology (ART[5]), specifically semen collection and analysis, are valuable for conducting detailed assessments of reproductive traits in felids, these techniques may be difficult to apply in the field with free-ranging cats, requiring some modification for practical *in situ* use [17]. Reproductive evaluations are an important component of managed breeding programs for felids in zoos, applying assisted reproductive techniques developed initially in domestic cats to their wild relatives [18–20]. In domestic cats, the most common methods used for semen collection are electroejaculation (EEJ) [16,21] and voluntary ejaculation using an artificial vagina (AV[6]) [22,23]. Although AV use has been reported for a single zoo-housed cheetah (*Acinonyx jubatus*) [24], this method is impractical for most free-ranging wild felids. In contrast, electroejaculation has been performed successfully in domestic cats for over 40 years and has been applied to virtually every non-domestic felid species [25–28] maintained in zoos, ranging in body size from the tiger (*Panthera tigris*) down to the black-footed cat (*Felis nigripes*) [28], as well as in free-ranging wild felids, including cheetahs, jaguars (*Panthera onca*) and lions (*Panthera leo*) [29]. Electroejaculation has been the method of choice in all wild cat species [12,15,30,31]; however, it can be challenging to apply in the field due to the need for costly equipment, species-specific rectal probes, and technical expertise. Furthermore, misconceptions about safety issues and possible tissue damage have hindered technique approval by ethical-scientific committees in some countries [27,32]. Broader application of semen collection and analysis for study and management of free-ranging wildlife populations, such as ocelots and bobcats, could benefit from development of simpler, more cost-effective semen recovery methods. In particular, a pharmaceutical-based approach for inducing sperm release directly into the urethra (without electrical stimulation) has been gaining credibility as an alternative semen collection method for felids and other mammalian species.

The use of pharmacologic agents for ejaculation and semen collection has been demonstrated over the past two decades in a variety of domestic species including horses [33–35], cats [36], and dogs [37]. One group of pharmaceuticals, alpha-2 adrenergic agonists (α_2 agonists[7]), are reported to influence erection [38,39] and the ejaculatory reflex [39] and cause contraction of smooth muscle within the vas deferens [40], forcing semen into the pelvic urethra [27]. Conveniently, field-based studies in non-domestic felids routinely use potent α_2 agonists, such as medetomidine or dexmedetomidine, in combination with other pharmaceutical agents for anesthesia.

Urethral catheterization (UC) of male cats following medetomidine sedation has allowed recovery of high sperm numbers in domestic cats [36], jungle cats (*Felis chaus*) [41], Amur leopard cats (*Prionailurus bengalensis*) [42], lions [43], and several other non-domestic felid species [44,45]. Due to the absence of seminal fluids, semen collected by UC usually has been characterized by a lower volume, higher sperm concentration, and lower pH compared to samples collected by EEJ. However, following semen freezing, post-thaw sperm parameters are similar for the two methods of collection [36]. Urethral catheterization conducted under medetomidine sedation, if proven to be equally effective as EEJ across cat species, may become the preferred semen collection method for global conservation efforts with imperiled wild felids.

Our objectives were to evaluate reproductive traits of free-ranging ocelots and bobcats in southern Texas for the first time to gain insight into their normative reproductive values while, concurrently, assessing the feasibility of UC with medetomidine-based anesthesia for semen collection of both species in a field setting. Study results will provide the first basal seminal data in wild ocelots and bobcats and possibly provide insight into the relationship of genetic diversity and reproductive traits in both species. Further, if semen collection by UC provides reliable seminal data from free-ranging ocelots, this approach may allow us to broaden our evaluations of other ocelot populations more easily.

2. Materials and methods

2.1. Animals

Wild male cats (n=31), representing 2 species from 2 genera (*Leopardus*: ocelot, n=9; *Lynx*: bobcat, n=22) were assessed in this study. Free-ranging felids were live-trapped using modified Tomahawk traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) on private ranches in Willacy and Kenedy counties in southern Texas and the Laguna Atascosa National Wildlife Refuge (LANWR) from December to April each year from December 2019 to May 2022. For most captures, a separate hardware cloth extension, measuring 51 cm×38 cm x 51 cm, was attached to the distal end of the trap to hold live prey (i.e., a pigeon or small chicken provided with water and feed *ad libitum*). Traps were checked each morning following sunrise to ensure evaluation and release of captured animals by early to mid-afternoon. All animal use was reviewed and approved by and in accordance with the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University-Kingsville (TAMUK), Caesar Kleberg Wildlife Research Institute (CKWRI), and the University of Tennessee- Knoxville. The study protocol also was reviewed for permitting under the Endangered Species Act by

[4] International Union for Conservation of Nature
[5] Assisted Reproductive Technology
[6] Artificial vagina
[7] Alpha 2 adrenergic agonists

the United States Fish and Wildlife Service (USFWS).

2.2. Semen collection and reproductive assessment

Male ocelots and bobcats were anesthetized and maintained at a light anesthetic plane for semen collection. The anesthetic protocol consisted of an injectable combination of ketamine hydrochloride (target dosage: ocelot, 4–8 mg/kg bodyweight; bobcat, 2.5–5 mg/kg bodyweight; Wedgewood Pharmacy: Wildlife Pharmaceuticals, Inc.; ZooPharm, Swedesboro NJ; and Henry Schein, Inc., Marlborough, MA) and medetomidine hydrochloride (target dosage: ocelot, 0.05 mg/kg bodyweight; bobcat, 0.06 mg/kg bodyweight; Wedgewood Pharmacy: Wildlife Pharmaceuticals, Inc. and ZooPharm, Swedesboro NJ) or dexmedetomidine (target dosage: 0.05 mg/kg bodyweight; Henry Schein, Inc., Marlborough, MA) given intramuscularly (IM) via pole syringe or hand injection followed by partial reversal with atipamezole (IM) (target dosage: 5 mg of atipamezole for every 1 mg of medetomidine given or 10 mg of atipamezole for every 1 mg of dexmedetomidine given; Wedgewood Pharmacy: Wildlife Pharmaceuticals, Inc.; ZooPharm, Swedesboro NJ; and Henry Schein, Inc., Marlborough, MA). Fasting prior to the anesthetic event was not possible due to the free-ranging nature of the felids; however no negative effects were noted. Body temperature, heart rate, and respiratory rate were monitored throughout the procedure for all felids; and pulse oximetry, jaw tone, and capillary refill time were additionally monitored in all but one of the procedures conducted at LANWR.

Both testicles were palpated by hand for firmness and recorded on a 1–3 scale: 1) hard 2) normal and 3) flaccid. Length and width of each testicle was measured in millimeters using calipers (Fig. 1), the volume of each testis (left, L, and right, R) was calculated using the following elliptical equation: length (L) X width $(W)^2$ X 0.524 (LTVOL; RTVOL) and volume of each added together for the total testicular volume (TTVOL, cm^3). Relative testicular weight (RTWT) was calculated as total testicular volume (cm^3) per kg of body weight. Approximately 20–40 minutes post anesthetic injection, the penis was extruded with manual manipulation using sterile gloves, debris removed with a water-soaked gauze and the glans penis examined for the presence or absence of penile spines (Fig. 1) and hygiene. A sterile 3.5 or 5 French (1.2 or 1.7 mm diameter, respectively) modified open-ended urinary catheter was lightly lubricated (HR Lubricating Jelly, HR Pharmaceuticals Inc., York PA) and advanced approximately 13–15 cm into the urethra (Fig. 1), left in place for 30 seconds and then slowly removed [36]. For most ocelots, if no ejaculate was recovered by UC or the sample was nonviable, one to three series of electroejaculation (EEJ) (2–5 V; 10 stimulations per series) were performed using a standardized technique [46] with slight modification in probe size, number of stimuli, and collection sets based on each species [47,48,51]. Electroejaculation was not applied during the first two years of the project timeline due to permitting constraints.

In the field, the recovered catheter sample was transferred into an Eppendorf vial using air pressure from a one ml syringe. Seminal volume (VOL) was measured using a micro-pipettor and an aliquot was initially assessed for the presence or absence of spermatozoa using microscopy (100X). For spermic samples, motility (percent progressively motile, PPM; 0–100 %) and rate of forward progressive motility (FPM; scale of 0–5, with 0 being non-motile and 5 being rapid forward progression) were evaluated microscopically (100X) using raw aliquots from each sample [46]. Urine contamination was determined by pH (≤6.5; ColorpHast # 9583, EMD Chemicals Inc. Darmstadt, Germany), discoloration, and low motility (<10 %) and rate of forward sperm progression (1/5). A subset of raw semen (1–3 μL) was fixed in 49 μl of 0.3 % glutaraldehyde and later assessed in the laboratory with phase contrast microscopy at 400X magnification (100–200 sperm/sample) to determine the percentage of spermatozoa with normal morphology (MORPH) (Fig. 2). Sperm morphology was documented on a Zeiss Axioskop Fluorescent microscope equipped with an AxioCam 202 monocolor camera using positive phase contrast 40X objective or a 100X objective with oil. An aliquot of raw semen was diluted in water (1:400) to determine sperm concentration (CONC) using a hemocytometer. Total sperm per ejaculate (TSE) was calculated by multiplying the VOL and CONC. Another aliquot (4 μl) of raw semen was spread onto a microscope slide and dried at ambient temperature in the field, and then later stained in the laboratory with fluorescein isothiocyanate-peanut agglutinin (Sigma-Aldrich Corporation, St. Louis, MO), and assessed with fluorescence microscopy (100–200 sperm/sample) to determine percentage of intact acrosomes (ACRO) [49]. Acrosome status was classified as intact, partially intact, or non-intact (Fig. 3); and was assessed using fluorescence (excitation 465–495/emission >515) on a Zeiss Axioskop Fluorescent microscope equipped with AxioCam ERc5s. All images were obtained and processed using Zen 3.1 (blue edition; Carl Zeiss Microscopy GmbH, Germany). The remaining raw semen from each male was diluted (1:1 – 1:5) in warmed Feline Optimized Culture

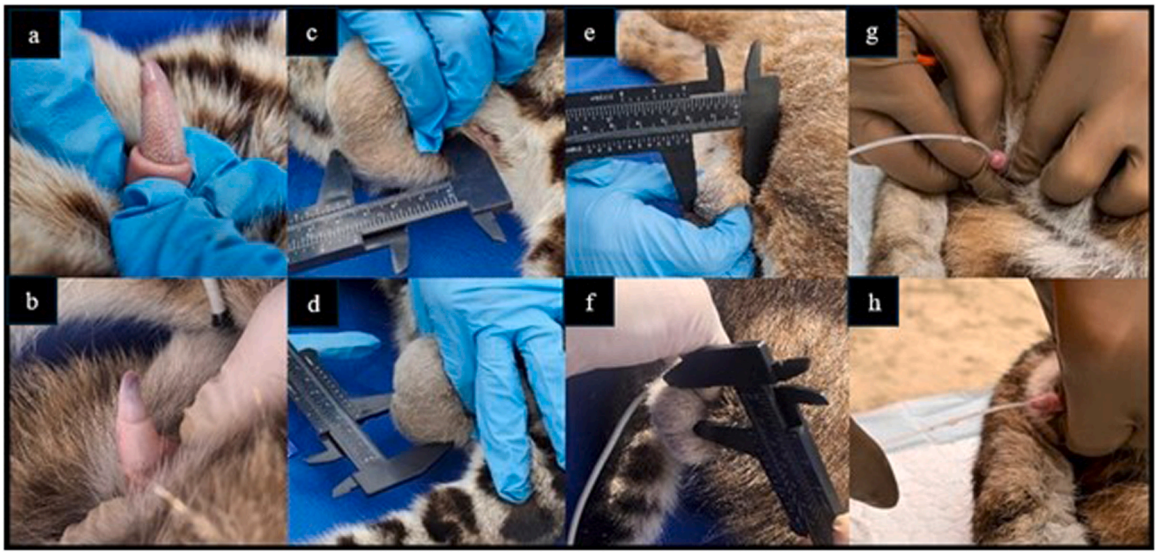

Fig. 1. Testicular parameters, penile morphology, and urethral catheterization in ocelots and bobcats in southern Texas from 2019 to 2022. (a) keratinized spines on the glans penis of an ocelot (*Leopardus pardalis*); (b) lack of keratinized spines on the glans penis of a bobcat (*Lynx rufus*); width and length of a testis as measured by calipers on an ocelot (c, d) and bobcat (e, f); (g, h) urethral catheterization of a bobcat.

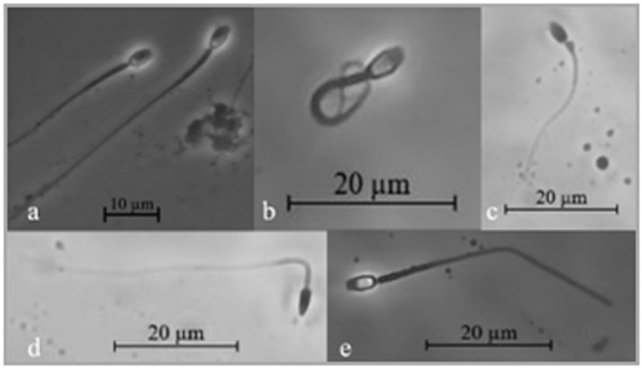

Fig. 2. Normal and abnormal sperm morphology documented in ocelots and bobcats in southern Texas from 2019 to 2022. (a) normal sperm morphology; primary abnormalities: (b) tightly coiled tail; and secondary abnormalities: (c) proximal droplet, (d) bent midpiece without a droplet, (e) bent tail.

Medium (FOCM) with HEPES [50] in the field and transported to the laboratory at ambient temperature for further processing for sperm cryopreservation (data not shown).

2.3. Fecal and serum testosterone analyses

2.3.1. Fecal and serum sample collection and processing

Fecal and serum samples were collected from wild individuals anesthetized for semen collection and physical exam. Fecal samples were collected after natural voiding within the trap or by rectal palpation with a lubricated gloved finger. Blood samples were collected using cephalic or jugular venipuncture and serum samples recovered from clot tubes post-centrifugation. Samples were transferred into cryovials and stored at −20 °C until processing. Fecal samples were lyophilized using a freeze dryer (Virtis, Gardener, NY, USA) in their cryovials, pulverized into a fine powder, and then weighed (250± 5 mg) into labeled 15 ml polypropylene conical tubes. Each of the samples was then extracted by adding 2.5 ml of 90 % ethanol (1:10 w:v) overnight on a mechanical rocker (≥12 h). Extracted samples were then centrifuged (1000 g, 15 min), supernatants were pipetted off and samples stored in 2.0 ml cryovials at −20 °C until analysis. Serum samples were thawed, diluted in diethyl ether at 1:5, vortexed for 30 seconds, and placed in a −80 °C freezer for 10 min. The ether was then poured off into separate tubes that were placed under a vacuum in a fume hood until dry (~ 1 h), and then allowed to sit overnight. The samples were reconstituted at a 1:1 dilution with EIA buffer (45.2 mM NaH2PO4, 61.0 mM Na2HPO4, 148 mM NaCl, 0.1 % BSA, 0.0001 % Proclin 150, 863.5 mM Tween 20) and stored in 2.0 ml cryovials at −20 °C until analysis.

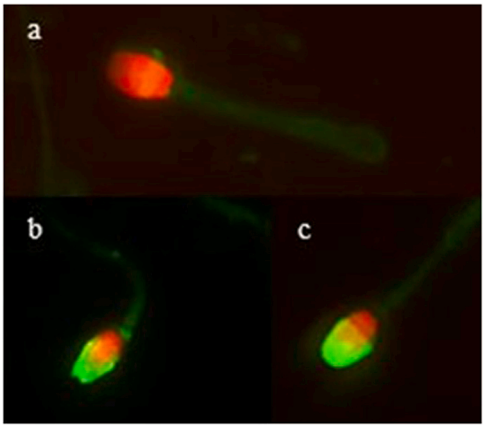

Fig. 3. Acrosomal staining and classification. (a) Non-intact acrosome. (b) Partially intact acrosome. (c) Intact acrosome.

2.3.2. Enzyme immunoassays

A testosterone assay kit (ISWE001, Arbor Assays Testosterone Minikit, Ann Arbor, MI) was used to determine androgen levels, following the standardized kit protocol with included antibody and horseradish peroxidase (HRP). Cross reactivities for anti-testosterone antibody were testosterone 100 %, dihydrotestosterone 35.4 %, progesterone 0.024 %, corticosterone <0.004, cortisol <0.004, cortisone <0.004, and 17β-estradiol <0.004.

2.3.3. Fecal testosterone analysis

For ocelot fecal analysis, small aliquots of extracted sample were assessed in the assay from 1:80 – 1:250 dilution as determined by the parallelism for fecal testosterone in ocelots (1:16 – 1:512, $R^2 = 0.999$). There was significant recovery (average of 74.4 %) of exogenous testosterone added to ocelot fecal extracts ($y = 0.99x+0.149$, $R^2 = 0.998$). An inter-assay coefficient of variation (CV) of equal to or less than 10 % was observed, with an intra-assay CV of 12.3 %. For bobcat fecal analysis, small aliquots of extracted sample were evaluated in the assay from 1:25–1:50 as determined by the parallelism for fecal testosterone in bobcats (1:4 – 1:512, $R^2 = 0.999$). There was significant recovery (average of 76.84 %) of exogenous testosterone added to bobcat fecal extracts ($y = 1.16x+0.22$, $R^2 = 0.999$). An inter-assay CV of ≤10 % and an intra-assay CV of 12.3 % was observed.

2.3.4. Serum testosterone analysis

Small aliquots (50 µl) of extracted serum samples were evaluated without further dilution in the testosterone assay as determined by parallelism for serum testosterone in ocelots (neat – 1:4, $R^2 = 0.999$). There was significant recovery (average of 78.75 %) of exogenous testosterone added to ocelot serum extracts ($y = 1.0x+0.139$, $R^2 = 0.998$), and (average of 76.25 %) for bobcats ($y = 1.0x+0.13$, $R^2 = 0.999$). The testosterone assay was incubated for 2 hours, plates were then run through a plate washer (AquaMax 2000, Molecular Devices, San Jose, CA, USA) with wash buffer (85.9 mM Na2HPO4*7H2O, 13.9 mM NaH2PO4*H2O, 150.06 mM NaCl, 12.73 mM EDTA, 0.09 % Proclin 150, 1.06 % Tween 20; diluted to 1X from 20X), and 3,3',5,5'-tetramethylbenzidine (TMB, Sigma-Aldrich, St. Louis, MO, USA) was added to each well. After ~10 minutes of development, a solution of 3 % hydrochloric acid (HCl) was added to each well to stop the reaction and the plate was then evaluated for optical density on a plate reader (VersaMax Absorbance microplate reader, Molecular Devices, San Jose, CA, USA) at 450 nm. Samples and standards were analyzed in duplicate. An inter-assay CV of ≤12.5 % and an intra-assay CV of 12.3 % was observed.

2.4. Statistical analyses

Normality was assessed using the Shapiro-Wilk test at a 95 % confidence interval. If a normal distribution was reported, the mean values ± SEM was reported in the results. If there was a non-normal distribution, the median (minimum-maximum) values were reported in the results. Mean anesthetic dosages, total testicular volumes, total testicular volumes to weight ratio, and mean ejaculate characteristics were calculated for both species. Non-parametric comparisons between species (for total testicular volumes, STEST, FTEST) were assessed using the Mann-Whitney U test for statistical significance. Parametric comparisons between species were assessed using independent samples t-tests (total testicular volume-to-weight ratios, percent normal morphology). Non-parametric correlations between medetomidine dosages and ejaculate traits were assessed using Spearman's rho correlation coefficient, whereas parametric correlations were evaluated using Pearson correlation coefficient. Independent sample t-tests were used to determine if urine contamination affected the percentage of normal sperm morphology. The effect of treatment (collection method) on various seminal traits was assessed using mixed model analysis (ANOVA) with a diagonal covariance structure and cat as a random factor for ocelots.

Seasonal effects were assessed using the Kruskal-Wallis test across months and Mann-Whitney test across season. All analyses were performed in SPSS 28 with an α= 0.05.

3. Results

3.1. Anesthesia and semen collection

From 2019–2022, 35 bobcats (22 males, 13 females) and 21 ocelots (9 males, 12 females) were captured, of which all captured males were included in this study. Mean ± SEM anesthetic dosages were as follows for each species: ocelot, 9.19 ± 1.01 (range 4.93–15.83) mg/kg ketamine, 0.062 ± 0.006 (range 0.031–0.1) mg/kg medetomidine, and 0.30 ± 0.042 (range 0.00–0.505) mg/kg atipamezole; bobcat, 4.72 ± 0.52 (range 2.63–11.98) mg/kg ketamine, 0.07 ± 0.002 (range 0.052–0.095) mg/kg medetomidine, and 0.036 ± 0.027 (range 0.00–0.722) mg/kg atipamezole. Urethral catheterization resulted in recovery of sperm samples from seven ocelots in nine attempts (78 %) and 14 bobcats in 22 attempts (64 %). Urine contamination occurred in 6 of 7 ocelot samples (86 %) and 6 of 14 bobcat samples (43 %). Of urine contaminated samples, two ocelot samples and no bobcat samples remained viable (i.e., retained ≥ 30 % motility). Electroejaculation resulted in recovery of semen from four of five ocelots (80 %) with urine contamination in two samples (50 %). Both of these urine-contaminated samples remained viable (i.e., retained ≥ 30 % motility) due to rapid dilution in culture medium, centrifugation (600xg for 8 minutes), and removal of the supernatant.

3.2. Reproductive traits

On physical exam, penile spines were documented in seven of nine (78 %) ocelots and zero of 22 (0 %) bobcats. No sperm was recovered with UC from the two ocelots lacking penile spines. These cats were estimated to be under two years of age and therefore reproductively immature. Unilateral cryptorchidism was observed in two bobcats with one bobcat also having a persistent penile frenulum (Appendix B: LB02M, EB31M; Fig. 4). Comparing cat species, total testicular volume (TTVOL) was greater (p<0.001) in ocelots (20.9 cm^3 (range 1.85–22.66) than in bobcats (2.37 cm^3 (range 0.47–5.25);) as was the total testicular volume-to-weight ratio (ocelots, 1.51 ± 0.21 cm^3/kg; bobcats, 0.29 ± 0.03 cm^3/kg; p<0.001). For samples collected by UC, there was no correlation in either species between total testicular volume and ejaculate volume (bobcat, rho= 0.165, p=0.51; ocelot, rho= 0.583, p=0.10), sperm concentration (bobcat, rho= 0.210, p=0.40; ocelot, rho= 0.214, p=0.61), and total sperm per ejaculate (bobcat, rho= 0.316, p=0.20; ocelot, rho= 0.238, p=0.57). Similarly, for EEJ samples in ocelots, total testicular volume was not correlated with ejaculate volume (rho= 0.389, p=0.52), sperm concentration (rho= 0.546, p=0.34), and total sperm per ejaculate (rho= 0.664, p=0.22).

Fig. 4. Unilateral cryptorchidism and persistent penile frenulum in a bobcat in southern Texas. Black circle = unilateral cryptorchidism, presence of only the left testicle; Black arrow = persistent penile frenulum.

Values for seminal traits are presented in Table 1. Urine contamination of sperm samples collected by UC in both species and EEJ in ocelots did not affect the percentage of normal morphology (RAW 27.8 ± 4.6, Urine Cont. 29.4 ± 5.0; p= 0.83), therefore urine contaminated samples were included in morphologic analysis. For ocelots, the percentage of primary morphologic abnormalities was higher (p<0.001) for UC (47.75 ± 6.7) compared to EEJ (9.00 ± 2.7) but the percent normal morphology (MORPH) did not differ between UC and EEJ (p=0.22) (Table 1). For both species, dilution of semen in FOCM-Hepes medium did not improve the percentage of normal morphology (RAW = 27.8 ± 4.6, FOCM = 30.4 ± 4.1; p=0.68), primary abnormalities (p=0.96), secondary abnormalities (p=0.75), or total abnormalities (p=0.54). The most common sperm abnormality for ocelots with UC collection was tightly coiled tails whereas bent tails predominated with EEJ collection. When urine contamination occurred, bent tails were the most common abnormality in ocelots, regardless of collection method. For bobcats, the most common sperm morphological abnormality was a tightly coiled tail (in 93 % of males), regardless of urine presence.

Urine contamination had no effect on percent sperm motility (p=0.26) and rate of forward progressive motility (p=0.12) for ocelots, but urine decreased percent motility [p=0.014; without urine 5.5 (0−40); with urine 0 (0−10)] and rate of forward progressive motility [p=0.013; without urine 3 (0−4); with urine 0 (0−3)] in bobcats.

For anesthesia, medetomidine dosage had no effect on total ejaculate volume (ocelot: rho= −0.067, p= 0.87; bobcat: rho= −0.064, p=0.80), sperm concentration (ocelot: rho= 0.048, p= 0.91; bobcat: rho= −0.142, p=0.57), or sperm per ejaculate (ocelot: rho= 0.190, p= 0.65; bobcat: rho= −0.243, p=0.33) for either species. When assessing seasonality, differences were examined at the month level (Dec through Apr) and seasonal level [Winter (December-February) and Spring (March-April)]. For ocelots, neither month (M) or season (S) affected the total ejaculate volume (M: p=0.13; S: p= 0.19), sperm concentration (M: p=0.42; S: p= 0.39), or sperm per ejaculate (M: p=0.44; S: p= 0.39). Of the 22 male bobcats and 9 male ocelots included in this study, the following were captured per month: December (4 bobcats, 1 ocelot), January (5 bobcats, 2 ocelots), February (0 bobcats, 2 ocelots), March (10 bobcats, 2 ocelots), and April (3 bobcats, 2 ocelots). For bobcats, there was an effect of both month and season on total ejaculate volume (M: p=0.019; S: p=0.001) and an effect of season on sperm concentration (p= 0.014) and total sperm per ejaculate (p=0.043). However, the month of collection did not affect either sperm concentration (p=0.08) or sperm per ejaculate (p=0.19). In bobcats, total ejaculate volume was higher in the winter (W) than spring (S) (W: 170 (38−320) μl; S: 29.3 (10−60) μl), whereas sperm concentration (W: 1 (0−56) X 10^6 sperm/ml; S: 31.5 (0.125–125.5) X 10^6 sperm/ml) and sperm per ejaculate (W:

Table 1
Mean (±SEM) and median (minimum-maximum) values for seminal traits in two free-ranging felid species in southern Texas captured from December to April each year from December 2019- May 2022.

	Ocelot UC (n=6)	Ocelot EEJ (n=4)	Bobcat UC (n=14)
VOL*	321.0 (0.9–968.0)	468.0 (200.0–922.0)	38.4 (10.0–320.0)
CONC*	124.0 (1.0–535.5)	29.5 (0.0–97.0)	16.0 (0.1–125.5)
TSE*	23.3 (0.7–269.5)	53.6 (0.0–152.5)	0.3 (0.0–36.8)
TMS*	3.8 (0.0–80.9)	42.9 (0.0–137.3)	0.03 (0.03–14.70)
ACRO	40.8 (± 13.3)	73.3 (± 5.0)	40.5 (± 4.4)
MORPH	36.9 (± 8.5)	58.1 (± 12.8)	23.3 (± 3.9)
PPM	50.0 (± 15.3)	83.8 (± 2.4)	14.8 (± 5.1)
FPM	3.3 (± 0.4)	3.9 (± 0.4)	2.4 (± 0.5)

UC, urethral catheterization collection technique; EEJ, electroejaculation collection technique; VOL, semen volume (μl); CONC, sperm concentration (x 10^6 / ml); TSE, total sperm per ejaculate (x 10^6); TMS, total motile sperm (x 10^6); ACRO, intact acrosome status (%); MORPH, normal sperm morphology (%); PPM, percent progressively motile (%); FPM, rate of forward progressive motility (0−5); SEM, Standard Error of the Mean. *values reported are median values (min-max) due to a non-normal distribution.

0.067 (0–3.08) X 10^6; S: 0.374 (0.003–36.8) X 10^6) were higher in the spring.

Serum and fecal testosterone concentrations (mean ± SEM) for each species were as follows: ocelot- serum (0.314 (0.05–0.67) ng/ml), fecal (239.32 (64.55–1406.25) ng/g); bobcat- serum (0.181 (0.063–0.660) ng/ml), fecal (147.74 (39.8–459.56) ng/g). There was no difference between species for serum testosterone (p=0.45) or fecal testosterone (p=0.26), nor any effect of season (winter vs spring) on fecal or serum testosterone for bobcats (fecal, p=0.25; serum, p=0.43) or ocelots (fecal, p=0.25; serum, p=0.43). There was no correlation between serum testosterone and fecal testosterone for ocelots (p=0.87) or bobcats (p=0.67). For EEJ samples in ocelots, serum testosterone was positively correlated with total sperm per ejaculate (rho=0.900, p=0.037) and total motile sperm (rho=0.900, p=0.037) but not with sperm concentration (rho=0.700, p=0.188). For UC samples in ocelots, there was no correlation between serum testosterone and sperm concentration (rho=-0.200, p=0.70), total sperm per ejaculate (rho=-0.107, p=0.82), and total motile sperm (rho=-0.036; p=0.94). Because of limited numbers of ocelot fecal samples, correlation of fecal testosterone with semen parameters could not be assessed. For UC collection in bobcats, neither serum testosterone or fecal testosterone correlated with sperm concentration (rho= 0.140, p=0.66; rho=-0.511, p=0.07, respectively), total sperm per ejaculate (rho= 0.196, p=0.54; rho=-0.236, p=0.44), or total motile sperm (rho= −0.342, p=0.45; rho=-0.485; p=0.19).

4. Discussion

This study represents the first detailed assessment of reproductive traits in wild ocelots and bobcats living within their natural habitats. All previous research has focused on evaluating wild-born or captive-born ocelots and bobcats maintained under human care in zoos, with indeterminate impacts of multiple husbandry factors on measured reproductive characteristics [25,26,51,52]. Of note, nine wild male ocelots were evaluated in the current study, possibly representing ~20 % of all males within the last extant breeding population in the US [53–55]. To assess seminal characteristics, we used a newer method of semen collection (urethral catheterization, UC) for both free-ranging ocelots and bobcats. Comparison of semen collection by urethral catheterization (UC) to electroejaculation (EEJ) in ocelots revealed potentially meaningful differences in ejaculate traits between methods. In bobcats, UC allowed recovery of a low-quality ejaculate with some differences observed based on seasonality. While ocelots produced higher quality ejaculates than bobcats, overall seminal quality in the wild ocelot population appeared slightly inferior to values reported for ocelot populations managed within zoos [25,27,51,56].

With UC in ocelots, recovered samples consisted of low ejaculate volumes containing highly concentrated spermatozoa with a relatively low pH, as observed in studies in other feline species [30,36,49,57–59]. One primary challenge with UC was urine contamination of the semen, occurring within the urethra prior to recovery, similar to findings in earlier reports [25,56]. UC samples typically lack seminal fluids so exposure to urine with acidic pH and variable osmolarity, coupled with delayed sample recovery while waiting on adequate anesthetic induction for catheterization, negatively impacts sperm viability even with immediate dilution in an isotonic medium post-recovery.

The addition of EEJ to the sampling protocol allowed recovery of higher sperm numbers with improved percent motility and rates of forward progression, as previously observed in ocelots [56]. Ocelot EEJ samples, while also frequently contaminated with urine, usually contain a greater volume of seminal fluid, and thus have a higher alkalinity and lower osmolarity, which may provide better buffering against urine. For bobcats, urine contamination occurred less often (about half the time) than in ocelots using the UC method, but the comparative effect of EEJ was not assessed. Further, a slight reduction in the depth of catheter insertion into the urethra (13–14 cm) relative to previously reported distances (ocelots, 15 cm; Canada lynx, 15 cm) [56,60] failed to decrease the occurrence of urine contamination in either species, suggesting inadvertent catheter insertion into the urinary bladder was not a factor.

For urethral catheterization, alpha-2-agonist drugs such as medetomidine and dexmedetomidine [36] are essential to cause sperm deposition into the urethra. In domestic cats, the dosage of medetomidine that yielded adequate sperm numbers was 0.13–0.14 mg/kg [30], or 1.5–2 times higher than dosages used in the present study, and greater than dosages (0.05–0.11 mg/kg) used in other felid studies [49,57,58]. With dexmedetomidine, semen collection by UC requires a much lower dosage (0.025 mg/kg) in domestic cats [15,27,61,62], compared to medetomidine to recover similar total sperm numbers. Medetomidine is a racemic mixture of 2 enantiomers, d-MED and l-MED, with d-MED (dexmedetomidine) being the active variant [63] displaying a higher binding affinity to α_2 receptors [64,65]. In other felid species, urine contamination was more likely to occur with EEJ under the influence of medetomidine as compared to dexmedetomidine [66]; although addition of supplemental anesthetics (isoflurane and propofol) [66] and electrical stimuli applied during EEJ also could promote subsequent urine release [43]. In the current study, a dose effect on sperm recovery was not observed with medetomidine. Increasing medetomidine dosages to 0.13–0.14 mg/kg (i.m.) in ocelots and bobcats could improve sperm yield [63], but also would heighten concerns of α_2 agonist-induced hemodynamic effects [67].

Our reproductive assessments in this study included evaluation of testicular dimensions and penile morphology. A large difference was observed between species in testes size, with ocelots having ~8 times the testicular volume of bobcats despite having similar body weights. Ocelots also exhibited higher total sperm numbers than bobcats, but within species, there was no correlation of total sperm recovery with testicular volume, which was consistent with earlier findings in wild felid species [51]. Although serum testosterone concentrations did not differ between species, ocelots displayed large, obvious penile spines, whereas bobcat penises were spineless. Penile spines in felids are androgen-dependent and thought to increase tactile stimulation for induced ovulation [68,69] and serve as holdfast organs, comparable to the bulbourethral gland in dogs [70,71]. Because ocelots are strictly induced ovulators with non-seasonal reproduction and relatively high androgen levels in males [51,72–74], the presence of penile spines in this species likely function for these stated purposes.

In contrast, bobcats are reported to be seasonal breeders, as are their closely related relatives in the *Lynx* genus [75,76], but it is unknown whether they are spontaneous or induced ovulators. In any case, the absence of penile spines in bobcats appears to be species-specific [51] rather than androgen dependent [77].

Results from our study support the occurrence of reproductive seasonality in bobcats and the absence of seasonality in ocelots (at least during the winter-spring months). Seminal parameters in wild ocelots did not differ from winter to spring, which is consistent with findings from an earlier study of zoo-housed ocelots [51]. In contrast, bobcats exhibited a greater ejaculate volume in the winter months with a higher sperm concentration and total sperm per ejaculate in the spring. Similar to other lynx species [75], bobcats appear to produce superior sperm numbers in the springtime but do not show any seasonality in serum and fecal testosterone levels, similar to that of zoo- managed bobcats [52] and Iberian lynx (*Lynx pardinus*) [78]. The opposite pattern was observed in the more temperate species, the Canada lynx (*Lynx canadensis*) [75] and Eurasian lynx (*Lynx lynx*) [76], which showed distinct seasonality in testosterone concentrations.

In our study, the comparison of UC and EEJ in ocelots revealed some apparent differences in reproductive parameters based on collection technique. Specifically, the percentage of primary sperm abnormalities increased with UC compared to EEJ. Sperm abnormalities in fresh, undiluted semen can result from defects in spermatogenesis (primary abnormalities) [79,80] or sperm transport through the excurrent duct system of the testis, after spermatogenesis (secondary abnormalities)

[79–81]. With EEJ and UC in ocelots, the most common abnormalities were bent tails (i.e., a secondary sperm abnormality) and tightly coiled tails (i.e., a primary sperm abnormality), respectively. However, when urine was present in the UC sample, the most common abnormality did not differ from EEJ collected samples, suggesting that a urine-related osmotic effect may have altered sperm morphology. Tightly coiled tails commonly occur with sperm exposure to hypo-osmolar solutions, which forms the basis for the hypo-osmotic swelling test (HOST) for assessing sperm membranes [82]. Accordingly, the absence of seminal fluid in UC samples may be partially responsible for a high percentage of coiled tails that subsequently decreased after exposure to the highly concentrated urine typical of ocelots. Because samples were collected in a field situation, methods for semen processing and transport also could affect seminal parameters.

One objective in this study was to compare seminal traits of wild Texas ocelots to that of ocelots managed under human care. For zoo-housed ocelots, EEJ resulted in recovery of 114.7 ± 15.8 $\times 10^6$ motile sperm per ejaculate with 82.4 ± 1.2 % morphologically normal sperm, and males averaged 1.71 ± 0.14 μg/g fecal androgens [51], with similar values reported in another zoo-based study [25]. When UC and EEJ were compared in zoo-housed ocelots, UC produced 36.2 ± 28.2 $\times 10^6$ sperm per ejaculate with 72.1 ± 11.5 % motility whereas EEJ produced 55.6 ± 22.7 $\times 10^6$ total sperm per ejaculate with 87.1 ± 4.9 % motility [56]. For samples collected by UC in wild Texas ocelots, the percent sperm motility and relatively high frequency of urine contamination were similar to these previous reports [25,56]. Although UC resulted in more primary sperm abnormalities compared to EEJ, other sperm parameters did not differ significantly, but total sperm numbers, sperm motility traits and normal sperm morphology tended to be higher in EEJ collected samples. In turn, our seminal values from EEJ of wild ocelots, including percent normal sperm morphology, tended to be lower than that observed with ocelots managed under optimal zoo conditions [25, 51,56], and more similar to findings from a broad-based reproductive survey of Latin American zoos [27], in which sample quality was impacted by sub-optimal diets and husbandry [27]. Because ocelots in the current study were free-ranging, we suspect that compromised semen quality was less likely the result of poor nutrition or other environmental factors, but possibly could be related to reduced genetic diversity. Unfortunately, no seminal data are available for comparison from any other wild, free-ranging ocelot population, including those having greater genetic heterozygosity. Abnormal physical traits previously associated with decreased heterozygosity and inbreeding in felids were not observed in the Texas ocelot population.

In free-ranging Texas bobcats, we observed similar testicular volume, percent normal morphology, and sperm concentrations to other species of the *Lynx* genus, but acrosome integrity and percent sperm motility were much lower [78]. Our samples from wild bobcats were recovered using UC whereas in Canada lynx, UC was ineffective and electroejaculation proved to be superior for semen collection. Similarly, EEJ of zoo-housed bobcats produced higher values for seminal volume, sperm concentration, total sperm per ejaculate, and percent motility [52]. Decreased genetic diversity in bobcats has been documented in the Lower Rio Grande Valley [9] and, we suspect that this reduced genetic variation coupled with the low reproductive potential typical of this genus might contribute to the sub-optimal semen quality seen within this wild population. In our study, unilateral cryptorchidism was observed in two individuals and UC collection failed to recover a spermic ejaculate in either male. Additionally, a persistent penile frenulum was seen in a single bobcat which may be associated with inbreeding [5, 83–88]. Additionally, a single ocelot male at LANWR was also observed with unilateral cryptorchidism; however a semen sample was unable to be recovered by UC due to excessive urination at the time of collection [unpublished data]. This male was not included in the present study but could suggest an effect of inbreeding depression and subsequent negative impacts to reproductive health.

Although the UC method allowed semen recovery from both species and was easier to apply in a field setting, our results suggest that EEJ remains the preferred collection method for characterization of normative seminal traits and maximizing sperm recovery for potential semen banking of wild males for conservation purposes. With UC collection, the lower values observed for sperm motility traits, acrosome status and morphology could negatively impact sperm transport and function within the reproductive tract, especially following cryopreservation, and ultimately, reduce reproductive capacity [46,68,69, 89–95]. Notably, our findings indicate that both wild ocelots and bobcats exhibited teratospermia (>60 % morphologically abnormal sperm) based on UC collection, although ocelots were nearly normospermic (>60 % normal morphology) in EEJ samples. Teratospermia is a trait commonly observed in non-domestic Felidae [4,5,12,13,15] with some teratospermic species also having reduced genetic variation and low circulating testosterone concentrations. If wild Texas ocelots are teratospermic, as suggested by UC results, this finding would heighten the risk that reduced genetic diversity is already affecting reproductive parameters in this species. In turn, this conclusion likely would increase the urgency to implement genetic augmentation to help stabilize the existing wild population into the future.

5. Conclusions

Urethral catheterization following medetomidine treatment was an effective method of sperm collection for characterization of basal seminal traits in free-ranging ocelots and bobcats; however, urine contamination was a consistent challenge that decreased the overall quality and viability of sperm samples. For ocelots, use of electroejaculation as a secondary collection technique allowed recovery of additional semen with improved traits and less impairment from urine contamination. With free-ranging ocelots, if the primary goal is to maximize total sperm recovery for cryopreservation, especially in males that may only be captured once in their lifetime, then electroejaculation should be considered as the first option. Further refinement of methodology may be warranted with either collection technique, including investigating methods to reduce or mitigate urine contamination, slightly increasing the medetomidine dosage to improve total sperm recovery or possibly assessing dexmedetomidine as an alternative as only one male was collected under dexmedetomidine treatment for this study. In free-ranging bobcats, further investigation should include comparing the effectiveness of EEJ to UC for field use in this species and defining seasonal influences on reproductive traits. Lastly, our findings suggest that both felid populations may be experiencing slight reduction in reproductive parameters relative to zoo-housed cats, possibly related to decreased gene diversity, but further research is necessary to fully evaluate this hypothesis. At a minimum, results of the current study provide a baseline of reproductive parameters in these free-ranging ocelot and bobcat populations that may be monitored over time to assess changes associated with genetic recovery or further population declines.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendices

A. Seminal traits for individual ocelots collected by urethral catheterization and electroejaculation in southern Texas from 2019 to 2022.

	WT (KG)	TTVOL	VOL	CONC	TSE	TMS	ACRO	MORPH	PPM	FPM
Urethral Catheterization										
E19M*	13.4	20.9	148	535.5	79.3	55.5	72	59.8	70	4
E22Mˆ*	11	20.3	408	3.67	9	7.2	44	35	80	1
Y27Mˆ*	13.8	21.4	79	294.9	23.3	14	77	21.8	60	2.5
Y28Mˆ	5.5	1.85	0							
E13Mˆ*	12.5	21.7	968	124	120	0	33	73	0	0
E24Mˆ*	9.8	8.1	663	1	0.7	0	7	15	0	0
OM341ˆ*	11.4	22.3	321	7.5	9.5	3.8	26	14	40	0.5
LO02M	5.7	0.95	43							
Electroejaculation										
E22Mˆ*	9.3	22.7	922	97	99.5	79.6	76	27	80	2
E13M*	12.5	21.7	250	8	7.6	6.1	60.5	69	80	3
E24M	9.8	8.1	200							
LO04Mˆ*	9.8	13.3	431	47	13.8	11.7	71	86.5	85	4.5
OM341*	11.4	22.3	686	51	152.5	137.3	85	50	90	4.5

TTVOL, total testicular volume (cm^3); VOL, semen volume (μl); CONC, sperm concentration (x 10^6 / ml); TSE, total sperm per ejaculate (x 10^6); TMS, total motile sperm (x 10^6); ACRO, intact acrosome status (%); MORPH, normal sperm morphology (%); PPM, percent progressively motile (%); FPM, rate of forward progressive motility (0−5); ˆurine contamination; *These samples were used for evaluation of spermic traits in Table 1.

B. Seminal traits for individual bobcats collected by urethral catheterization in southern Texas from 2019 to 2022.

	WT (kg)	TTVOL	VOL	CONC	TSE	TMS	ACRO	MORPH	PPM	FPM
EB31M#	10	2.46								
EB33M*	9.09	2.44	25	5.5	0.137	0.04	42	28.5	30	3
EB34Mˆ*	8.4	1.97	10	42.5	0.425	0.004	14.5	38.5	1	3
EB35Mˆ	8	1.48	1000				17	50		
EB36M*	8.1	2.21	54.4	56.5	3.08	0.03	21	5.3	1	4
EB37M*	11.8	5.16	36.8	40	1.47	0.59	31	19.5	40	4
EB38Mˆ	11.8	3.8	200							
EB39Mˆ	9.09	1.08	191							
EB42M*	9.78	2.64	60	58.5	3.51	0.35	64	27.5	10	3
EB44Mˆ	6.93	2.95	1000				24	27.5		
EB45M*	6.81	2.04	14	23	0.32	0.003	60	28.8	1	3
YB7Mˆ	10.23	1.01	1000				15	19		
YB9Mˆ*	9.99	4.25	33.6	125.5	4.22		18	9.5		
YB10Mˆ*	9.27	3.91	1000				28.5	9		
EB48M*	8.24	2.86	73	1	0.73		47	37		
EB50Mˆ*	7.96	1.85	40	2.12	0.25	0.03	38	19	10	1.5
EB51Mˆ	6.9	0.54	50	0.125	0.003					
EB53M*	9.44	2.29	15	122.5	36.8	14.7	70	29	40	1
EB54M*	9.16	1.56	14	9	0.126	0.01	53	31	10	3
LB01Mˆ*	8	5.15	320	1	0.067		33	12.5		
LB02M#	8.7	0.47	0							
LB03Mˆ*	9.66	5.25	170	2	0.34		35	25		

TTVOL, total testicular volume (cm3); VOL, semen volume (μl); CONC, sperm concentration (x 106 / ml); TSE, total sperm per ejaculate (x 106); TMS, total motile sperm (x 106); ACRO, intact acrosome status (%); MORPH, normal sperm morphology (%); PPM, percent progressively motile (%); FPM, rate of forward progressive motility (0−5); #unilateral cryptorchid; ˆurine contamination; *These samples were used for evaluation of spermic traits in Table 1.

References

[1] J.E. Janečka, M.E. Tewes, L.L. Laack, L.I. Grassman Jr., A.M. Haines, R. Honeycutt, Small effective population sizes of two remnant ocelot populations (*Leopardus pardalis albescens*) in the United States, Conserv Genet 9 (4) (2008) 869–878, https://doi.org/10.1007/s10592-007-9412-1.

[2] J.E. Janečka, M.E. Tewes, L.L. Laack, A. Caso, L.I. Grassman Jr, A.M. Haines, D. B. Shindle, B.W. Davis, W.J. Murphy, R.L. Honeycutt, Reduced genetic diversity and isolation of remnant ocelot populations occupying a severely fragmented landscape in southern Texas, Anim. Conserv 14 (6) (2011) 608–619. ⟨https://doi-org.utk.idm.oclc.org/10.1111/j.1469-1795.2011.00475.x⟩.

[3] J.E. Janecka, M.E. Tewes, L. Laack, A. Caso, L.I. Grassman Jr., R.L. Honeycutt, Loss of genetic diversity among ocelots in the United States during the 20th Century linked to human induced population reductions, Plos One 9 (2) (2014) e89384, https://doi.org/10.1371/journal.pone.0089384.

[4] D.E. Wildt, M. Bush, K.L. Goodrowe, C. Packer, A.E. Pusey, J.L. Brown, P. Joslin, S. J. O'Brien. Reproductive and genetic consequences of founding isolated lion populations, Nature 329 (6137) (1987) 328, https://dx.doi.org/10.1038%2F329328a0.

[5] M.E. Roelke, J.S. Martenson, S.J. O'Brien, The consequences of demographic reduction and genetic depletion in the endangered Florida panther, Curr. Biol. 3 (1993) 340–350, https://doi.org/10.1016/0960-9822(93)90197-v.

[6] P.W. Hedrick, S.T. Kalinowski, Inbreeding depression in conservation biology, Annu Rev. Ecol. Syst. 3 (1) (2000) 139–162, https://doi.org/10.1146/annurev.ecolsys.31.1.139.

[7] D.H. Reed, Relationship between population size and fitness. Relación entre Tamaño Poblacional y Adaptabilidad, Conserv Biol. 19 (2) (2005) 563–568. ⟨https://doi-org.utk.idm.oclc.org/10.1111/j.1523-1739.2005.00444.x⟩.

[8] D.H. Reed, A.C. Nicholas, G.E. Stratton, Genetic quality of individuals impacts population dynamics, Anim. Conserv 10 (3) (2007) 275–283. ⟨https://doi-org.utk.idm.oclc.org/10.1111/j.1469-1795.2007.00120.x⟩.

[9] J.E. Janecka, M.E. Tewes, I.A. Davis, A.M. Haines, A. Caso, T.L. Blankenship, R. L. Honeycutt, Genetic differences in the response to landscape fragmentation by habitat generalist, the bobcat, and a habitat specialist, the ocelot, Conserv Genet 17 (2016) 1093–1108, https://doi.org/10.1007/s10592-016-0846-1.

[10] S. Larivière, L.R. Walton, Lyns rufus, Mamm. Species 563 (1997) 1–8.

[11] M. Kelly, D. Morin, C.A. Lopez-Gonzalez, Lynx rufus, IUCN Red. List Threat. Species (2016) e.T12521A50655874. https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T12521A50655874.en. (Accessed on 06 January 2022).

[12] D.E. Wildt, M. Bush, J.G. Howard, S.J. O'Brien, D. Meltzer, A. Van Dyk, H. Ebedes, D.J. Brand, Unique seminal quality in the South African cheetah and a comparative evaluation in the domestic cat, Biol. Reprod. 29 (4) (1983) 1019–1025, https://doi.org/10.1095/biolreprod29.4.1019.

[13] D.E. Wildt, S.J. O'Brien, J.G. Howard, T.M. Caro, M.E. Roelke, M. Bush, Similarity in ejaculate-endocrine characteristics in captive versus free-ranging cheetahs of two subspecies, Biol. Reprod. 36 (2) (1987) 351, https://doi.org/10.1095/biolreprod36.2.351.

[14] S.J. O'Brien, D.E. Wildt, D. Goldman, C.R. Merril, M. Bush, The cheetah is depauperate in genetic variation, Science 221 (1985) 459–462, https://doi.org/10.1126/science.221.4609.459.

[15] D.E. Wildt, L.G. Phillips, L.G. Simmons, P.K. Chakraborty, J.L. Brown, J.G. Howard, A comparative analysis of ejaculate and hormonal characteristics of the captive male cheetah, tiger, leopard and puma. *Biol Reprod.* 38 (1988) 245–255, https://doi.org/10.1095/biolreprod38.2.245.

[16] J.G. Howard, J.L. Brown, M. Bush, D.E. Wildt, Teratospermic and normospermic domestic cats: ejaculate traits, pituitary-gonadal hormones, and improvement of spermatozoal motility and morphology after swim-up processing, J. Androl. 11 (3) (1990) 204–215. ⟨https://doi-org.utk.idm.oclc.org/10.1002/j.1939-4640.1990.tb03229.x⟩.

[17] W.F. Swanson, Application of assisted reproduction for population management in felids: the potential and reality for conservation of small cats, Therio 66 (2006) 49–58, https://doi.org/10.1016/j.theriogenology.2006.03.024.

[18] E. Axnér, C. Linde-Forsberg Semen collection and assessment, and artificial insemination in the cat, in: P. Concannon et al., Recent advances in small animal reproduction, International *Veterinary Information Service*. (2002) 1-9.

[19] D. Zambelli, S. Belluzzi, Collection and evaluations of cat seminal material, Raccolta e valutazione del materiale seminale di gatto, Prax. Vet. 1 (1998) 20–22.

[20] D. Zambelli, M. Cunto, Semen collection in cats: Techniques and analysis, Therio 66 (2) (2006) 159–165, https://doi.org/10.1016/j.theriogenology.2006.01.054.

[21] E.S.E. Hafez. Reproduction and breeding techniques for laboratory animals. Philadelphia, Lea & Febiger: Philadelphia, 1970.

[22] M.P. Dooley, M.H. Pineda, J.G. Hopper, W.G. Hsu, Retrograde flow of spermatozoa into the urinary bladder of cats during electroejaculation, collection of semen with an artificial vagina, and mating, Am. J. Vet. Res 52 (5) (1991) 687–691, https://doi-org.utk.idm.oclc.org/10.1016/0093-691X(94)90502-A.

[23] N.J. Sojka, L.L. Jennings, C.E. Hamner, Artificial insemination in the cat (*Felis catus L.*), Lab Anim. Care 20 (1970) 198–204. PMID: 4246013.

[24] B.S. Durrant, T. Schuerman, S. Millard, Noninvasive semen collection in the cheetah, Proc. A Mtg Am. Assoc. Zoo. Pks Aquar. (1985) 564–567.

[25] M.A. Stoops, J.B. Bond, H.L. Bateman, M.K. Campbell, G.P. Levens, T.R. Bowsher, S.T. Ferrell, W.F. Swanson, Comparison of different sperm cryopreservation procedures on post-thaw quality and heterologous *in vitro* fertilization success in the ocelot (*Leopardus pardalis*), Reprod. Fertil. Dev. 19 (2007) 685–694, https://doi.org/10.1071/RD06078.

[26] W.F. Swanson, W.E. Johnson, R.C. Cambre, S.B. Citino, K.B. Quigley, D. M. Brousset, R.N. Morais, N. Moreira, S.J. O'Brien, D.E. Wildt, Reproductive status of endemic felid species in Latin American zoos and implications for *ex situ* conservation, Zoo. Biol. 22 (2003) 421–441. ⟨https://doi-org.utk.idm.oclc.org/10.1002/zoo.10093⟩.

[27] W.F. Swanson, H.L. Bateman, L.M. Vansandt, Urethral catheterization and sperm vitrification for simplified semen banking in felids, Reprod. Domest. Anim. 52 (2) (2016) 255–260, https://doi.org/10.1111/rda.12863.

[28] L.M. Vansandt, H.L. Bateman, A.G. Miller, J.R. Herrick, A. Moresco, R. Gonzalez, M.E. Iwaniuk, W.F. Swanson, Cross-species efficacy of a chemically-defined, soy lecithin-based cryomedium for semen banking in imperiled wild felids, Therio 159 (2021) 108–115. ⟨https://doi-org.utk.idm.oclc.org/10.1016/j.theriogenology.2020.10.021⟩.

[29] A.E. Crosier, L. Marker, J. Howard, B.S. Pukazhenthi, J.N. Henghali, D.E. Wildt, Ejaculate traits in the Namibian cheetah (*Acinonyx jubatus*): influence of age, season and captivity, Reprod. Fertil. Dev. 19 (2007) 370–382, https://doi.org/10.1071/rd06057.

[30] F. Göritz, K.M. Neubauer, S.V. Naidenko, J. Fickel, K. Jewgenow, Investigations on reproductive physiology in the male Eurasian lynx (*Lynx lynx*). Therio 66 (6) (2006) 1751–1754. ⟨https://doi-org.utk.idm.oclc.org/10.1016/j.theriogenology.2006.02.036⟩.

[31] D. Jayaprakash, S.B. Patil, M.N. Kumar, K.C. Majumdar, S. Shivaji, Semen Characteristics of the Captive Indian Leopard, Panther pardus. J. Androl. 22 (1) (2001) 25–33. ⟨https://doi-org.utk.idm.oclc.org/10.1002/j.1939-4640.2001.tb02149.x⟩.

[32] E. Furthner, N. Cordonnier, M. Le Dudal, A. Fontbonne, V. Freiche, Is electroejaculation a safe procedure in cats? An endoscopic and histological prospective blinded study, Therio 119 (2018) 69–75, https://doi.org/10.1016/j.theriogenology.2018.06.013.

[33] S.M. McDonnell, Oral imipramine and intravenous xylazine for pharmacologically-induced ex copula ejaculation in stallions, Anim. Reprod. Sci. 68 (2001) 153–159, https://doi-org.utk.idm.oclc.org/10.1016/S0378-4320(01)00152-X.

[34] T.M.S. Calvero, F.O. Papa, R.A.M. Schmith, V.F.C. Scheeren, L.E.F. Canuto, M.L. M. Gobato, L.T. Rodrigues, C.P. Freitas-Dell'aqua. Protocols using detomidine and oxytocin induce ex copula ejaculation in stallions, Therio 140 (2019) 93–98, https://doi.org/10.1016/j.theriogenology.2019.08.024.

[35] C.E. Card, S.T. Manning, P. Bowman, T. Leibel, Pregnancies from imipramine and xylazine-induced ex copula ejaculation in a disabled stallion, Can. Vet. J. 38 (3) (1997) 171–174. ⟨https://www-ncbi-nlm-nih-gov.utk.idm.oclc.org/pubmed/9056069⟩.

[36] D. Zambelli, F. Prait, M. Cunto, E. Iacono, B. Merlo, Quality and in vitro fertilizing ability of cryopreserved cat spermatozoa obtained by urethral catheterization after medetomidine administration, Therio 69 (4) (2008) 485–490. ⟨https://doi-org.utk.idm.oclc.org/10.1016/j.theriogenology.2007.10.019⟩.

[37] A.H. Kuczmarski, M. Alves de Barros, L.F. Souza de Lima, T.F. Motheo, H.J. Bent, G.A. Iglesias, D.A. Sonego, R.C. Rodrigues da Paz, Urethral catheterization after pharmacological induction for semen collection in dog, Therio 53 (2020) 34–38. ⟨https://doi-org.utk.idm.oclc.org/10.1016/j.theriogenology.2020.04.035⟩.

[38] S. Silinski, C. Walzer, F. Schwarzenberger, L. Slotta-Bachmayr, R. Stolla, Pharmacological methods of enhancing penile erection for ex-copula semen collection in standing white rhinoceros (*Ceratotherium simum simum*). Eur. Assoc. Zoo. Wildl. Vet. (EAZWV) 4th Sci. Meet. (2002).

[39] E.S. Hafez, Anatomy of male reproduction, in: B. Hafez, E.S.E. Hafez (Eds.), Reprod Farm Anim, Lippincot Williams and Wilkins, Maryland, 2013, pp. 3–11.

[40] A. Macdonald, J.C. McGrath, The distribution of adrenoceptors and other drug receptors between the two ends of the rat vas deferens as revealed by selective agonists and antagonists, Br. J. Pharm. 71 (2) (1980) 445–458, https://doi.org/10.1111%2Fj.1476-5381.1980.tb10957.x.

[41] M.S. Kheirkhah, M. Mollapour sisakht, M. Mohammadsadegh, H.R. Moslemi, Sperm evaluation of Jungle Cat (*Felis chaus*) obtained by urethral catheterization (CT) after medetomidine administration, Therio 91 (2017) 17–20. ⟨https://doi-org.utk.idm.oclc.org/10.1016/j.theriogenology.2016.12.034⟩.

[42] D.H. Jeong, J.H. Kim, K.J. Na, Characterization and cryopreservation of Amur leopard cats (*Prionailarus bengalensis euptilurus*) semen collected by urethral catheterization. *Therio* 119 (2018) 91–95, https://doi.org/10.1016/j.theriogenology.2018.06.004.

[43] I. Lueders, I. Luther, G. Scheepers, G. van der Horst, Improved semen collection method for wild felids: urethral catheterization yields high sperm quality in African lions (*Panthera leo*). Therio 78 (3) (2012) 696–701. ⟨https://doi-org.utk.idm.oclc.org/10.1016/j.theriogenology.2012.02.026⟩.

[44] G.R. Araujo, T.A.R. Paula, T. Deco-Souza, R.G. Morato, L.C.F. Bergo, L.C.D. Silva, D.S. Costa, C. Braud, Comparison of semen samples collected from wild and captive jaguars (*Panthera onca*) by urethral catheterization after pharmacological induction, Anim. Reprod. Sci. 195 (2018) 1–7, https://doi.org/10.1016/j.anireprosci.2017.12.019.

[45] P.N. Jorge-Neto, G.R. Araujo, T. Deco-souza, R.F. Bittencourt, A.C. Csermak Jr, C. S. Pizzutto, M.C.C. Silva, J.A. Salomão Jr, M. Madrigal-Valverde, V.P. Curvelo, M. C. Gomes, T.A.R. Paula, Pharmacological semen collection of Brazilian wild felids, Rev. Bras. Reprodução Anim. 43 (2019) 704.

[46] J.G. Howard, D.E. Wildt. Semen collection, analysis and cryopreservation in nondomestic mammals, In: Morrow D.A., editor. Current therapy in theriogenology: diagnosis, treatment, and prevention of reproductive diseases in small and large animals, Saunders, 2nd edition. (1986) 1047-1053.

[47] J.R. Herrick, M. Campbell, G. Levens, T. Moore, K. Benson, J. D'Agostino, G. West, D.M. Okeson, R. Coke, S.C. Portacio, K. Leiske, C. Kreider, P.J. Polumbo, W. F. Swanson, In vitro fertilization and sperm cryopreservation in the black-footed cat (*Felis nigripes*) and sand cat (*Felis margarita*), Biol. Reprod. 82 (2010) 552–562, https://doi.org/10.1095/biolreprod.109.081034.

[48] K. Thiangtum, W.F. Swanson, J.G. Howard, W. Tunwattana, D. Tongthainan, W. Wichasilpa, P. Patumrattanathan, T. Pinyopoommintr, Assessment of basic seminal characteristics, sperm cryopreservation and heterologous in vitro fertilisation in the fishing cat *(Prionailurus viverrinus)*. Reprod. Fertil. Dev. 18 (3) (2006) 373–382, https://doi.org/10.1071/rd05098.

[49] M.M. Vick, H.L. Bateman, C.A. Lambo, W.F. Swanson, Improved cryopreservation of domestic cat sperm in a chemically defined medium, Therio 78 (9) (2012) 2120–2128, https://doi.org/10.1016/j.theriogenology.2012.08.009.

[50] J.R. Herrick, J.B. Bond, G.M. Magarey, H.L. Bateman, R.L. Krisher, S.A. Dunford, W.F. Swanson, Development of a feline optimized culture medium: effects of ions, carbohydrates, essential amino acids, vitamins and serum on the development and metabolism of IVF-derived feline embryos relative to embryos grown in vivo, Biol. Reprod. 76 (2007) 858–870, https://doi.org/10.1095/biolreprod.106.058065.

[51] R.N. Morais, R.G. Mucciolo, M.L.F. Gomes, O. Lacerda, W. Moraes, N. Moreira, L. H. Graham, W.F. Swanson, J.L. Brown, Seasonal analysis of semen characteristics, serum testosterone and fecal androgens in the ocelot (*Leopardus pardalis*), margay (*L. wiedii*) and tigrina (*L. tigrinus*), Therio 57 (8) (2002) 2027–2041, https://doi.org/10.1016/s0093-691x(02)00707-0.

[52] A. Gañán, R. González, A. Sestelo, J.J. Garde, I. Sánchez, J.M. Aguilar, M. Gomendio, E.R.S. Roldan, Male reproductive traits, semen cryopreservation, and heterologous in vitro fertilization in the bobcat (*Lynx rufus*), Therio 74 (2009) 341–352, https://doi.org/10.1016/j.theriogenology.2009.03.002.

[53] J.E. Janečka, M.E. Tewes, L.L. Laack, L.L. Grassman, Jr, A.M. Haines, R. Honeycutt, Small effective population sizes of two remnant ocelot populations (*Leopardus pardalis albescens*) in the United States, Conserv Genet 9 (4) (2008) 869–878, https://doi.org/10.1007/s10592-007-9412-1.

[54] A.M. Haines, J.E. Janecka, M.E. Tewes, L.L. Grassman Jr, P. Morton, The importance of private lands for ocelot (*Leopardus pardalis*) conservation in the United States, Oryx 40 (1) (2006) 90–94, https://doi.org/10.1017/S0030605306000044.

[55] A.M. Haines, M.E. Tewes, L.L. Laack, W.E. Grant, J. Young, Evaluating recovery strategies for an ocelot (*Leopardus pardalis*) population in the United States, Biol. Conser 126 (4) (2005) 512–522, https://doi.org/10.1016/j.biocon.2005.06.032.

[56] W.F. Swanson, L.M. Vansandt, S. Citino, R.S. Larsen, G.A. Cole, A. Moresco, Addressing the challenge of do-it-ourself (DIY) semen banking in wild felids, Proc. Ann. Meet. Am. Assoc. Zoo. Vet. (2017) 81–82.

[57] E.C. Feldman, R.W. Nelson. Canine and feline endocrinology and reproduction, third ed., Elsevier, Saunders, St. Louis, (2004).

[58] J. Howard, Semen collection, analysis and cryopreservation in nondomestic mammals, Curr. Ther. Therio (1986) 1047–1053.

[59] D. Zambelli, R. Raccagni, M. Cunto, G. Andreani, G. Isani, Sperm evaluation and biochemical characterization of cat seminal plasma collected by electroejaculation and urethral catheterization, Therio 74 (8) (2010) 1396–1402, https://doi.org/10.1016/j.theriogenology.2010.06.011.

[60] W.F. Swanson, Semen banking of wild felids for zoo vets, in: R.E. Miller, N. Lamberski, P. Calle (Eds.), Fowler's Zoo and Wild Animal Medicine Current Therapy, Volume 10, Elsevier, St. Louis, MO, 2022, pp. 773–780.

[61] M.C. Pisu, P. Ponzio, C. Rovella, M. Baravalle, M.C. Veronesi, Usefulness of an injectable anaesthetic protocol for semen collection through urethral catheterization in domestic cats. J. Feline Med Surg. (10) (2017) 1087–1090, https://doi.org/10.1177/1098612x16679589.

[62] M. Madrigal-Valverde, R.F. Bittencourt, A.D.L.R. Filho, V.F. Barbosa, C.A. Vieira, E. A. Romão, I.B. Barneiro, M.C. Azevedo, G.R. Araujo, Quality of domestic cat semen collected by urethral catheterization after the use of different α 2-adrenergic agonist, J. Feline Med Surg. 23 (2021) 745–750, https://doi.org/10.1177/1098612x20973183.

[63] R. Aantaa, A. Kallio, R. Virtanen, Dexmedetomidine, a novel alpha-2-adrenergic agonist. A review of its pharmacodynamic characteristics. Drugs of the Future, J. Feline Med Surg. 18 (1989) 49–56, https://doi.org/10.1177/1098612x20973183.

[64] R. Virtanen, J.M. Savola, V. Saano, L. Nyman, Characterization of the selectivity, specificity and potency of medetomidine as an alpha 2-adrenoceptor agonist, Eur. J. Pharm. 150 (1-2) (1988) 9, https://doi-org.utk.idm.oclc.org/10.1016/0014-2999(88)90744-3.

[65] M. Granholm, B.C. McKusick, F.C. Westerholm, J.C. Aspegrén, Evaluation of the clinical efficacy and safety of dexmedetomidine or medetomidine in cats and their reversal with atipamezole, Vet. Anaesth. Analg. 33 (2006) 214–223, https://doi.org/10.1111/j.1467-2995.2005.00259.x.

[66] J.C. Marrow, M. Woc-Colburn, L.A.C. Hayek, L. Marker, S. Murray, Comparison of two α_2-adrenergic agonists on urine contamination of semen collected by electroejaculation in captive and semi-free-ranging cheetah (*Acinonyx jubatus)*, J. Zoo. Wildl. Med 46 (2) (2015) 417–420, https://doi.org/10.1638/2014-0211r2.1.

[67] N. Romagnoli, D. Zambelli, M. Cunto, c Lambertini, D. Ventrella, M.B. Toaldo, Non-invasive evaluation of the haemodynamic effects of high-dose medetomidine in healthy cats for semen collection, J. Feline Med Surg. 18 (2016) 337–343, https://doi.org/10.1177%2F1098612X15583345.

[68] W.F. Swanson, T.L. Roth, E. Blumer, S.B. Citino, D. Kenny, D.E. Wildt, Comparative cryopreservation and functionality of spermatozoa from the normospermic jaguar (*Panthera onca*) and teratospermic cheetah (*Acinonyx jubatus*). Therio 45 (1) (1996), 241-241. ISSN:0093-691X.

[69] E.C. Feldman, R.W. Nelson. Canine and Feline Endocrinology and Reproduction. Third ed. St. Louis: St. Louis: Elsevier: Saunders. (2004).

[70] A. Nuhn. Lehrbuch der vergleichenden Anatomie, part 1. Caerl Winter, Heidelberg. 1886.

[71] E. Retterer, A. Lelièvre, Effects de la castration sur le chat, C. R. Soc. Biol. 73 (1912) 184–186.

[72] N. Moreira, E.L.A. Monteiro-Filho, W. Moraes, W.F. Swanson, L.H. Graham, O. L. Pasquali, M.L.F. Gomes, R.N. Morais, D.E. Wildt, J.L. Brown, Reproductive steroid hormones and ovarian activity in felids of the *Leopardus* genus, Zoo. Biol. 20 (2001) 103–116, https://doi.org/10.1002/zoo.1010.

[73] E. Mondolfi, Notes on the biology and status of the small wild cats in Venezuela, in: S.D. Miller, D.D. Everett (Eds.), Cats of the World: biology, conservation and management, National Wildlife Federation, Washington, D.C., 1986, pp. 85–124.

[74] R. Eaton, Breeding biology and propagation of the ocelot (*Leopardus [Felis] pardalis*), Zool. Gart. 1 (1977) 72–81.

[75] K.V. Fanson, N.C. Wielebnowski, T.M. Shenk, W.J. Jakubas, J.R. Squires, J. R. Lucas, Patterns of testicular activity in captive and wild Canada lynx (*Lynx canadensis*), Gen. Comp. Endocrin 169 (3) (2010) 210–216, https://doi.org/10.1016/j.ygcen.2010.09.001.

[76] M.N. Erofeeva, E.V. Pavlova, A.L. Antonevich, S.V. Naidenko, Seasonal changes in activity of males' reproductive system in Eurasian lynx, Russ. J. Theriol. 13 (1) (2014) 9–16, https://doi.org/10.15298/rusjtheriol.13.1.02.

[77] L.R. Aronson, M.L. Cooper, Penile spines of the domestic cat: their endocrine-behavior relations, Anat. Rec. 157 (1967) 71–78.

[78] N. Gañan, A. Sestelo, J.J. Garde, F. Martinez, A. Vargas, I. Sánchez, M.J. Pérez-Aspa, J.V. López-Bao, F. Palomares, M. Gomendio, E.R.S. Roldan, Reproductive traits in captive and free-ranging males of the critically endangered Iberian lynx (*Lynx pardinus*), Soc. Reprod. Fertil. (2010), https://doi.org/10.1530/REP-09-0259.

[79] S.W.J. Seager, Artificial insemination in dogs, in: T.J. Burke (Ed.), Small Animal Reproduction and Infertility, 1986, pp. 207–217.

[80] R. Payan-Carreira, S. Miranda, W. Nizanski. Artificial insemination in dogs. In: Milad Manafi (ed): Artificial insemination in farm animals. (2011).

[81] W.V. Holt, Epididymal origin of a coiled-tail sperm defect in a boar, J. Reprod. Fertil. 64 (2) (1982) 485–489, https://doi.org/10.1530/jrf.0.0640485.

[82] S. Prochowska, W. Niżański, A. Fontbonne, Hypo-osmotic swelling test (HOST) for feline spermatozoa: the simplified procedure and the aspect of sperm morphology, Animals 12 (2022) 903, https://doi.org/10.3390/ani12070903.

[83] R. González, A. Moresco, A. Miller, H. Bateman, L. Vansandt, D. Dembiec, A. Ista, W.F. Swanson, Assessment of semen traits in servals (*Leptailurus serval*) and Canada lynx (*Lynx canadensis*), Reprod. Fert. Dev. 31 (1) (2018) 176–177, https://doi.org/10.1071/RDv31n1Ab101.

[84] S.E. Romagnoli, Canine cryptorchidism, Vet. Clin. N. Am. Small Anim. Pratt (1991) 535–544.

[85] Burns M., Fraser M.N. (Eds): Genetics of the dog. Edinburgh: Oliver and Boyd; (1964).

[86] J.H. Claxton, N.T.M. Yeates, The inheritance of cryptorchidism in a small, crossbred flock of sheep, J. Hered. 63 (1972) 141–144, https://doi.org/10.1093/oxfordjournals.jhered.a108254.

[87] H.C. McPhee, S.S. Buckley, Inheritance of cryptorchidism in swine, J. Hered. 25 (1934) 295–303, https://doi.org/10.1093/oxfordjournals.jhered.a103951.

[88] S.J. Kogan. Clinical Pediatric Urology, 2nd edn., Saunders, New York, 1985.

[89] M.A. Barone, M.E. Roelke, J. Howard, J.L. Brown, A.E. Anderson, D.E. Wildt, Reproductive fitness of the male Florida panther: Comparative studies of *Felis concolor* from Florida, Texas, Colorado, Chile, and North American Zoos, J. Mammal. (1993), https://doi.org/10.2307/1382247.

[90] D.R.P. Tulsiani, A. Abou-Haila, Biological processes that prepare mammalian spermatozoa to interact with an egg and fertilize it, Scientifica (2012) https://doi.org/10.6064%2F2012%2F607427.

[91] J.G. Howard, A.M. Donoghue, L.A. Johnston, D.E. Wildt, Zona pellucida filtration of structurally abnormal spermatozoa and reduced fertilization in teratospermic cats, Biol. Reprod. 49 (1) (1993) 131, https://doi.org/10.1095/biolreprod49.1.131.

[92] J.G. Howard, M. Bush, D.E. Wildt, Teratospermia in domestic cats compromises penetration of zona-free hamster ova and cat zonae pellucidae, J. Androl. 12 (1) (1991) 36–45, https://doi.org/10.1002/j.1939-4640.1991.tb00211.x.

[93] H. Krzanowska, The passage of abnormal spermatozoa through the utero-tubal junction of the mouse, J. Reprod. Fertil. 38 (1974) 81–90, https://doi.org/10.1530/jrf.0.0380081.

[94] A. Nestor, M.A. Handel, The transport of morphologically abnormal sperm in the female reproductive tract of mice, Gamete Res 10 (1984) 119–125, https://doi.org/10.1002/mrd.1120100204.

[95] R.G. Saacke, J.H. Bame, D.S. Karabinus, K.J. Mullins, S. Whitman, Transport of abnormal spermatozoa in the artificially insemination cow based upon accessory spermatozoa in the zona pellucida, Proc. Inr Congr. Anim. Reprod. Artif. Insemin. 3 (1988) 292.