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Teratospermic and Normospermic Domestic Cats: Ejaculate Traits, Pituitary-Gonadal Hormones, and Improvement of Spermatozoal Motility and Morphology After Swim-Up Processing

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Electroejaculate traits, testicular volume, and circulating FSH, LH, and testosterone concentrations were compared between two populations of domestic cats consistently producing either a high (> 60%, normospermic) or low (< 40%, teratospermic) incidence of structurally normal spermatozoa/ejaculate. The effects of semen dilution in Biggers, Whitten, and Whittingham (BWW) or modified Krebs Ringer bicarbonate (mKRB) medium and swim-up processing on sperm viability and duration of motility *in vitro* also were assessed. Ejaculate volume, percent sperm motility, sperm progressive motility, motile spermatozoa/ejaculate, testes volume, and mean serum FSH and LH concentrations were similar ($P > 0.05$) between normospermic and teratospermic cats. However, sperm concentration/ml of ejaculate was greater and circulating testosterone levels were lower in teratospermic males. Swim-up processing increased ($P < 0.05$) percent sperm motility, progressive motility, and the number of structurally normal sperm cells recovered and also prolonged the duration of sperm motility in both cat populations. In teratospermic ejaculates, swim-up separation increased the proportion of morphologically normal spermatozoa recovered by more than two-fold. Diluting cat semen with either BWW or mKRB increased flagellar bending in both normospermic and teratospermic cats. The sperm motility characteristics of only the teratospermic ejaculates were influenced by medium type; mKRB increased percent sperm motility and progressive motility whereas BWW had no effect. Compared with undiluted raw ejaculates, the duration of sperm motility was improved 18- to 24-fold by diluting semen in either BWW or mKRB medium followed by swim-up processing. This study demonstrates that the electroejaculate characteristics of domestic cats vary markedly and that some males consistently produce high proportions of morphologically abnormal spermatozoa. Diminished serum testosterone concentrations and normal pituitary secretion of FSH and LH in teratospermic males suggest that there is an inverse relationship between gonadal androgen production and pleiomorphic spermatozoa in the domestic cat. The swim-up procedure is effective for recovering motile, structurally normal spermatozoa from teratospermic cats.

Key words: domestic cat, sperm morphology, teratospermia, swim-up, FSH, LH, testosterone.

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The domestic cat is an important animal model for studying heritable human diseases (Migaki, 1982) and comparative genetics (O'Brien, 1986). The management and propagation of cats in a laboratory environment often is complicated (Schmidt, 1986), and difficulties may be due to a poor understanding of reproductive physiology. The estrous cycle and the phenomenon of induced ovulation in the female cat have been studied in detail (Wildt et al, 1981; Shille et al, 1983), yet the data base on seminal traits and endocrine profiles in the male cat is limited. Viable spermatozoa have been collected from the ductus deferens or cauda epididymidis (Bowen, 1977; Niwa et al, 1985), and ejaculates have been obtained by either an artificial vagina (AV) (Platz et al, 1978) or electroejaculation (Carter et al, 1984). Ejaculates obtained by an AV usually have a smaller volume and greater sperm concentration than those obtained by electroejaculation, but percent sperm motility and forward progressive motility are unaffected by the method of semen collection (Platz et al, 1978). Undiluted cat spermatozoa are sensitive to environmental conditions and when exposed to a 37 C or 23 C room atmosphere, exhibit motility for only 60 or 140 min, respectively (Goodrowe et al, 1989). Motility duration is prolonged by diluting semen in tissue culture medium (Bowen, 1977; Goodrowe et al, 1989) and maintaining the diluted ejaculate at 23 C rather than 37 C (Goodrowe et al, 1989).

Systemic luteinizing hormone (LH) and testosterone concentrations have been measured in male cats (Johnson and Gay, 1981; Goodrowe et al, 1985; Sirett et al, 1986), however, circulating levels of follicle stimulating hormone (FSH) have not been reported for either gender. Baseline serum LH levels vary among adult, intact males (Goodrowe et al, 1985), and concentrations are similar to values observed in adult estrous females (Chakraborty et al, 1979). Systemic testosterone concentrations fluctuate among domestic cats and generally decrease markedly within individual males subjected to an acute bleeding interval of ~ 84 min (Carter et al, 1984).

The proportion of structurally abnormal spermatozoa/ejaculate in most laboratory cats is approximately 29% (Wildt et al, 1983); recently, however, we observed that certain males routinely produce more than 60% aberrant sperm forms. The identification of such cats is important as these males can serve as models for studying the impact of teratospermia, a condition common to many nondomestic and frequently rare Felidae species (Wildt et al, 1983; 1987a,b; Howard et al, 1984). The etiology of teratospermia in the domestic cat is unknown, but structurally defective spermatozoa observed in certain wild felids such as the cheetah (Wildt et al, 1983, 1987a) and geographically-isolated lion (Wildt et al, 1987b) have been related to parallel observations of diminished genetic variation and decreased circulating testosterone concentrations.

The aim of this study was to characterize ejaculate traits and endocrine profiles, including FSH, LH, and testosterone concentrations, in domestic cats producing high or low proportions of morphologically abnormal spermatozoa. The influence of aberrant forms on sperm viability was assessed following dilution in various culture media, centrifugation, and seminal plasma removal. To develop an approach for improving sperm morphology, the swim-up separation technique was evaluated for its usefulness in the recovery and isolation of structurally normal motile spermatozoa.

Materials and Methods

Semen Collection, Blood Sampling, and Testicular Measurements

Animals from two populations of adult, domestic cats producing either high (> 60%/ejaculate; normospermic; n = 3) or low (< 40%/ejaculate; teratospermic; n = 3) proportions of structurally normal spermatozoa were studied simultaneously from March through July. Cats were housed under similar conditions in the same room in individual, stainless-steel cages (83 cm high × 78 cm wide × 91 cm deep) containing a metal shelf (78 cm wide × 21 cm deep). Dry, commercial cat food (Purina Cat Chow, Ralston Purina Co, St Louis, MO) and water were available *ad libitum*. Each animal was exposed to approximately 12 h of natural daylight per day, 8 of these h being supplemented with artificial fluorescent illumination. For electroejaculation, each male was anesthetized with an intramuscular injection of ketamine-HCl (Vetalar®, Parke-Davis, Morris Plains, NJ, 25 mg/kg). Following induction of a surgical plane of anesthesia, the dimensions of each testis were measured (length and width in cm) using laboratory calipers. The latter measurements were used to calculate testis volume using the conversion formula for a prolate spheroid ($V = 4/3\ \pi\ ab^2$, where "a" is one-half the length and "b" is one-half the width, Harrison et al, 1977). A combined total testes volume/animal was determined as the sum of the right and left testis volume (Harrison et al, 1977). A blood sample (6–7 ml) was collected by jugular venipuncture immediately before onset of each electroejaculation procedure (Pre-EE) and immediately after electroejaculation (Post-EE). The interval between ketamine-HCl injection and the Pre-EE blood sample averaged 11.3 ± 1.1 min (± SEM), and the interval from Pre-EE to Post-EE averaged 28.8 ± 1.8 min. Blood samples were centrifuged at 1 h after collection and the sera stored at −20 C until analyzed for hormones.

For electroejaculation, a Teflon rectal probe (1 cm in diameter × 13 cm length) with three longitudinal, stainless-steel electrodes (2.6 mm in width and 3.75 cm in length) and an AC 60-Hz sine wave electroejaculator with a variable transformer were used to deliver the electrical stimuli (P.T. Electronics, Boring, OR). The probe was lubricated and inserted 7–9 cm into the rectum and the electrodes positioned ventrally. A sterile 5 ml plastic collection vial (Nalge Co, Division of Sybron Corp, Rochester, NY) was placed over the penis and gentle cranial pressure applied to expose the penis. To permit comparative analysis, a standardized electroejaculation regimen consisting of a total of 80 electrical stimuli given at the same voltage increments was administered to each male at 1–2 wk intervals (12 ejaculates/male). The total sequence was divided into three series consisting of 30 (10 stimulations at 2, 3 and 4 volts: Series 1), 30 (10 stimulations at 3, 4 and 5 volts: Series 2), and 20 (10 stimulations at 4 and 5 volts: Series 3) stimuli, respectively. The stimulus cycle constituted approximately 1 second from 0 to the desired voltage, 2 to 3 seconds at the desired voltage, and an abrupt return to 0 volts for 3 seconds. Each animal was rested for 2–3 min between series. A total of 72 ejaculates (36 from the normospermic cats; 36 from the teratospermic cats) was collected.

Semen Analysis and Processing

Ejaculate aliquots from all three series were combined and evaluated immediately at 250 × magnification using phase-contrast microscopy for subjective estimates of the percentage of motile spermatozoa and spermatozoal progressive motility. Progressive motility was classified as the type of forward progressive movement of the cell based on a scale of 0 = no movement to 5 = steady, rapid forward progression (Howard et al, 1986). At least four separate fields at 250 × were examined and an average percent motility and progressive motility rating calculated. To determine an overall spermatozoal assessment rating with equal emphasis on both spermatozoal percent motility and progressive motility, a spermatozoal motility index (SMI) was calculated: SMI = (spermatozoal progressive motility × 20) + (% spermatozoal motility) ÷ 2. Ejaculate volume was measured, and sperm concentration/ml of ejaculate was determined using a standard hemacytometer counting procedure (Howard et al, 1986). Motile spermatozoa/ejaculate ($\times 10^6$) was calculated in each individual by multiplying ejaculate volume times spermatozoal concentration/ml times % spermatozoal motility. Sperm morphology was evaluated by fixing ejaculate aliquots (20 μl) in 1% glutaraldehyde (Wildt et al, 1983; Howard et al, 1983, 1986) followed by phase-contrast microscopic examination of 200 spermatozoa/aliquot at 1000 ×. Cells were classified as normal or as having one of the following anomalies: macrocephalic, microcephalic, bicephalic, tricephalic, mitochondrial sheath aplasia (including partial or complete aplasia of mitochondrial sheath), tightly coiled flagellum, bent midpiece with or without cytoplasmic droplet, bent flagellum with or without cytoplasmic droplet, proximal or distal cytoplasmic droplet.

Samples containing spermatozoa with at least a 70% motility rating were transferred to 1.5 ml conical tubes (Sarstedt Inc, Princeton, NJ) and slowly diluted with either 200 μl Biggers, Whitten and Whittingham (BWW) medium containing 3 mg/ml bovine serum albumin (BSA) (Biggers et al, 1971) or modified Krebs Ringer bicarbonate (mKRB) medium containing 4 mg/ml BSA (Niwa et al, 1985).

Diluted semen was split into three aliquots: diluted raw (DR), non-swim-up (NS), and swim-up (SU). The NS and SU aliquots were centrifuged (300g, 8 min); the supernatant was discarded; 50 μl of fresh medium was layered gently onto each sperm pellet without disturbing the pellet. In the NS aliquot, the spermatozoa were immediately resuspended in the layered supernatant; in the SU aliquot, spermatozoa were allowed to migrate into the fresh layer of medium. After a 1 h incubation at room temperature (25 C), an aliquot of each of the three sperm suspensions was evaluated for percent sperm motility, progressive motility, concentration, and morphology.

Hormonal Assessment

A heterologous double-antibody radioimmunoassay (RIA) previously validated for the domestic cat (Chakraborty et al, 1979) was used to measure LH concentration. Rabbit anti-bovine antiserum was provided by Dr. J. J. Reeves (JJR # 5), and highly purified ovine LH (LER-1056-C2, used as iodinated tracer) and canine LH (LER-1685-1, used as standard) were provided by Dr. L. E. Reichert, Jr. Recovery estimates after subtracting endogenous serum LH from the assay of 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 ng canine LH added to domestic cat serum were 0.27, 0.46, 1.08, 2.16, 3.55, and 7.80 ng ($y = 0.96x + 0.04$; $r = 0.99$; $P < 0.001$). Inhibition curves for serum pools were parallel to the canine standards and the assay sensitivity was 1.5 ng/ml. All samples were analyzed in a single radioimmunoassay which had an intra-assay coefficient of variation of 12.8% (n = 6).

A double-antibody radioimmunoassay, previously developed for measuring bovine FSH (Acosta et al, 1983) and adapted for nondomestic felids (Brown et al, 1988), was validated for the domestic cat. Rabbit anti-ovine FSH was provided by Dr. J. A. Dias (JAD-LER 178), highly purified ovine FSH (LER-1976-A2, used as the iodinated tracer) was provided by Dr. L. E. Reichert, Jr., and ovine FSH (NIH-FSH-S8) served as the standard. Cross-reactivity was < 3% for 200 ng LH (NIH-LH-S18), growth hormone (NIH-GH-S11), prolactin (NIH-PRL-S12), and GnRH. Recovery estimates after subtracting endogenous serum FSH from the assay of 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, and 100.0 ng ovine FSH added to cat serum were 1.28, 3.32, 5.65, 12.9, 26.0, 53.1, and 97.1 ng ($y = 0.98x + 0.64$; $r = 0.99$; $P < 0.001$). Serial dilutions of serum pools were parallel to the standard curve using NIH-FSH-S8. Sensitivity was 5.0 ng/ml, and the intra-assay coefficient of variation was 3.7% (n = 7).

Testosterone was measured using a double-antibody radioimmunoassay ^{125}I kit (Radioassay Systems Laboratory, Carson, CA). This assay has been used previously in domestic cats (Carter et al, 1984; Goodrowe et al, 1985). The first and second antibodies were rabbit anti-testosterone-19-carboxymethyl-ether-BSA and goat anti-rabbit gamma globulin, respectively. Recovery estimates after subtracting endogenous serum testosterone from the assay of 0.05, 0.125, 0.25, 0.5, 1.25, and 2.5 ng

testosterone added to domestic cat serum were 0.07, 0.12, 0.25, 0.49, 1.01, and 2.68 ng ($y = 1.04x + 0.04$; $r = 0.99$; $P < 0.001$). The cross-reactivity characteristics of the first antibody were: 100% with testosterone; 10.3% with 5α-dihydrotestosterone; < 1.0% with 5α-dihydrotestosterone, 6β-hydroxytestosterone, androstenedione, androsterone, androstanedione, estrone, estradiol-17β, estriol, progesterone, and corticosterone. Inter- and intra-assay coefficients of variation were 8.9% (n = 6) and 2.4% (n = 7), respectively, and the minimum detectable assay sensitivity was 0.1 ng/ml.

Duration of Spermatozoal Motility

To determine the effects of dilution, centrifugation, seminal plasma removal, and swim-up separation on duration of sperm motility *in vitro*, a total of 36 ejaculates was processed and evaluated. On 12 occasions, semen was obtained within a 1 h interval from all males in either the normospermic or teratospermic groups and pooled within group (three ejaculates/trial; six normospermic trials, six teratospermic trials). A 50 μl aliquot was removed from the pooled sample, designated as undiluted raw (UR), and assessed for sperm motility and progressive motility at 0 h. The remaining volume was diluted with 600 μl of either BWW or mKRB medium, split into the three aliquots described previously (diluted raw, DR; swim-up, SU; non-swim-up, NS) and assessed for sperm viability (time, 0 h). After centrifugation (300g, 8 min) of the SU and NS aliquots, the supernatant from each was removed, and fresh medium (150 μl) was layered onto each sperm pellet. In the SU aliquot, spermatozoa were allowed to migrate into the medium in contrast to resuspension of the pellet in the NS aliquot. After a 1 h incubation at room temperature (25 C), each sample of UR, DR, NS, and SU spermatozoa was assessed for sperm motility. To standardize sperm concentration during the incubation period, each sample was diluted to a final concentration of 10×10^6 motile spermatozoa/ml. Sperm suspensions were transferred to a 5% CO_2 in air, humidified environment (37 C) and evaluated hourly for 5 h. A sperm motility index (SMI) rating was calculated for each estimate of sperm viability.

TABLE 1. *Seminal traits, testicular volume, and incidence of morphologically normal and abnormal spermatozoa in normospermic and teratospermic domestic cats*

	Normospermic ejaculates (n = 18)	Teratospermic ejaculates (n = 18)
Ejaculate volume (μl)	124.1 ± 9.5*	97.4 ± 9.5*
Sperm concentration (× 10^6/ml)	167.6 ± 43.6*	361.3 ± 43.6†
Sperm motility (%)	84.4 ± 5.9*	73.3 ± 5.9*
Sperm progressive motility	4.2 ± 0.3*	3.7 ± 0.3*
Sperm motility index	84.4 ± 5.2*	73.6 ± 5.2*
Motile spermatozoa/ ejaculate (× 10^6)	17.0 ± 3.8*	24.6 ± 3.8*
Testicular volume (cm^3)	4.3 ± 0.6*	3.8 ± 0.6*
Normal spermatozoa (%)	71.6 ± 6.4*	33.8 ± 6.4†
Abnormal spermatozoa (%)		
macrocephalic	0.1 ± 0.03	0.2 ± 0.03
microcephalic	0.1 ± 0.02	0.2 ± 0.02
bicephalic	0.2 ± 0.2	2.1 ± 0.2
tricephalic	0.0	0.8 ± 0.5
mitochondrial sheath aplasia	0.0	0.4 ± 0.1
tightly coiled flagellum	2.1 ± 1.6	5.3 ± 1.6
bent midpiece with droplet	9.3 ± 3.5	24.1 ± 3.5
bent midpiece without droplet	1.0 ± 1.3	2.3 ± 1.3
bent flagellum with droplet	0.9 ± 6.2	5.4 ± 6.2
bent flagellum without droplet	5.8 ± 7.0	8.2 ± 7.0
proximal cytoplasmic droplet	2.2 ± 1.5	5.1 ± 1.5
distal cytoplasmic droplet	6.7 ± 2.0	12.1 ± 2.0

*† Within rows, means ± pooled SEM with different superscripts differ ($P < 0.05$).

Statistical Analysis

Data were examined on the basis of a split-split plot design with the whole plot designated as the spermatozoal morphology population (normospermic and teratospermic groups), the sub-plot being medium (BWW and mKRB), and the sub-sub plot being sperm treatment (UR, DR, NS, and SU groups). Data were analyzed using a general linear models program (SAS, 1986). The dependent variable, % normal spermatozoa, was analyzed using raw and arc-sin transformed data. Because statistical analysis revealed no interpretive difference between the raw and transformed data, results are presented as least squares means ± standard error of the mean (SEM) of raw values. When a significant F value was calculated ($P < 0.05$), differences among sperm treatment means were determined by the preplanned comparisons using the least significant difference (LSD) multiple-comparison procedure. Comparisons within each sperm morphology population included differences between sperm treatments within each medium and between both media. In assessing hormonal data, a split plot experimental design was used where the whole plot was the sperm morphology population (normospermic and teratospermic groups) and the sub-plot was the time of collection during an electroejaculation (EE) procedure (Pre-EE and Post-EE). Differences were determined by the preplanned comparisons using the LSD procedure. Each SEM was based on pooled variances.

Results

Semen Analysis

Ejaculate volume, percent sperm motility, progressive motility, motility index, and testicular volume were similar ($P > 0.05$) between the normospermic and teratospermic cats (Table 1). Sperm concentration/ml of ejaculate was greater ($P < 0.05$) in teratospermic cats, however, the total number of motile spermatozoa/ejaculate was comparable ($P > 0.05$) between populations.

Consistent differences ($P < 0.01$) were observed in sperm morphology between the two cat groups.

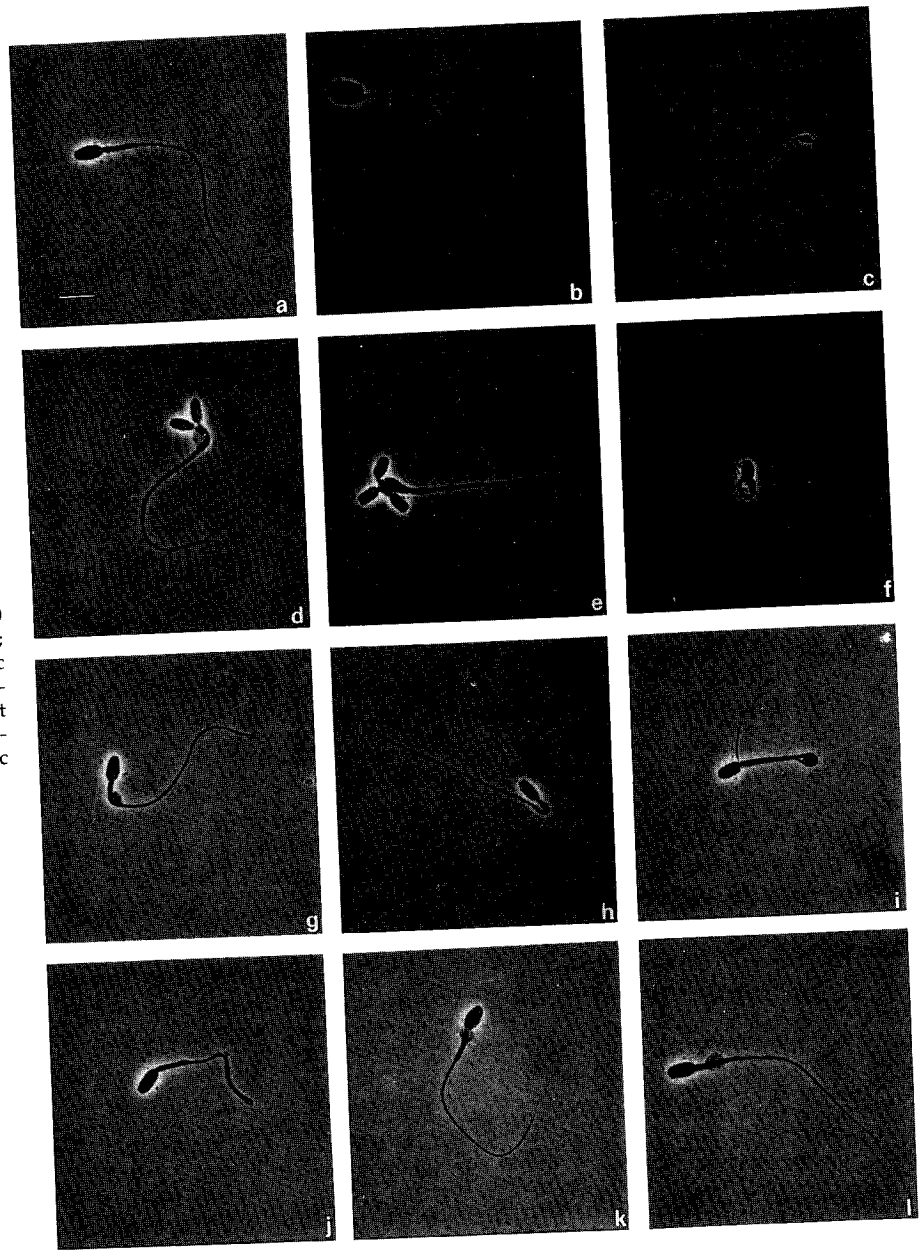

Fig. 1. Sperm forms detected in cat electroejaculate: (a) normal; (b) macrocephalic; (c) microcephalic with mitochondrial sheath aplasia; (d) bicephalic; (e) tricephalic; (f) tightly coiled flagellum; (g) bent midpiece with cytoplasmic droplet; (h) bent midpiece without cytoplasmic droplet; (i) bent flagellum with cytoplasmic droplet; (j) bent flagellum without cytoplasmic droplet; (k) proximal cytoplasmic droplet; (l) distal cytoplasmic droplet. Scale bar on Fig. 1a = 10 μm.

Examples of specific defects in sperm structure are depicted in Fig. 1. Compared with normospermic semen samples, teratospermic ejaculates in general contained a higher ($P < 0.05$) incidence of each type of sperm abnormality (Table 1). The most prevalent anomaly in the former group involved a bent midpiece with a laterally displaced cytoplasmic droplet positioned within the bent angle. The predominant abnormality in the teratospermic samples included spermatozoa with a bent midpiece with droplet, residual cytoplasmic droplet (proximal or distal), a bent flagellum (with or without a droplet), and a tightly coiled flagellum. In teratospermic males, sperm head defects (including macro- or microcephaly and bi- or tricephaly) affected 3.3% of all cells compared with only 0.4% in normospermic ejaculates.

Seminal traits including percent sperm motility, progressive motility, motility index, and structural morphology were influenced ($P < 0.01$) by processing treatment. In normospermic cats (Table 2), diluting the raw ejaculate (DR) or resuspending the ejaculate without swim-up processing (NS) reduced ($P < 0.05$) sperm motility ratings and the number

TABLE 2. *Influence of dilution, centrifugation, and swim-up separation on sperm viability and structural morphology in normospermic domestic cat ejaculates*

Normospermic ejaculates	Sperm treatment§			
	Undiluted raw	Diluted raw	Non-swim-up	Swim-up
Sperm motility (%)	84.4 ± 1.2*	76.4 ± 1.2†	75.3 ± 1.2†	90.8 ± 1.2‡
Sperm progressive motility	4.2 ± 0.1*	3.7 ± 0.1†	3.9 ± 0.1†	4.4 ± 0.1*
Sperm motility index	84.4 ± 1.1*	75.1 ± 1.1†	76.3 ± 1.1†	89.0 ± 1.1‡
Normal spermatozoa (%)	71.6 ± 2.3*	43.9 ± 2.3†	41.9 ± 2.3†	65.9 ± 2.3*
Abnormal spermatozoa (%)				
macrocephalic	0.1 ± 0.04	0.0	0.1 ± 0.04	0.0
microcephalic	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.0
bicephalic	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.0
tricephalic	0.0	0.0	0.0	0.0
mitochondrial shealth aplasia	0.0	0.0	0.0	0.0
tightly coiled flagellum	2.1 ± 0.8	3.5 ± 0.8	4.0 ± 0.8	0.9 ± 0.8
bent midpiece with droplet	9.3 ± 1.8	10.3 ± 1.8	9.0 ± 1.8	6.2 ± 1.8
bent midpiece without droplet	1.0 ± 0.5	3.7 ± 0.5	3.8 ± 0.5	2.7 ± 0.5
bent flagellum with droplet	0.9 ± 1.5	7.5 ± 1.5	8.0 ± 1.5	3.5 ± 1.5
bent flagellum without droplet	5.8 ± 1.8	29.7 ± 1.8	31.7 ± 1.8	18.9 ± 1.8
proximal cytoplasmic droplet	2.2 ± 0.3	0.2 ± 0.3	0.5 ± 0.3	0.3 ± 0.3
distal cytoplasmic droplet	6.7 ± 1.6	1.0 ± 1.6	0.8 ± 1.6	1.6 ± 1.6

*†‡ Within rows, means with different superscripts differ ($P < 0.05$).
§ Values are means ± pooled SEM of combined media treatment groups (BWW and mKRB).
Each value is based on 18 ejaculates from three normospermic cats (six ejaculates/male).

of structurally normal spermatozoa within 1 h of initial dilution. The latter resulted from marked increases in spermatozoa with a bent flagellum or midpiece defect. Increased flagellar bending occurred not only in structurally normal cells but also in spermatozoa afflicted with proximally- and distally-located cytoplasmic droplets. In a similar fashion, the DR and NS treatments also decreased ($P < 0.05$) sperm motility ratings and increased ($P < 0.05$) pleiomorphisms in teratospermic cats as well as the incidence of cells with a tightly coiled flagellum (Table 3).

TABLE 3. *Influence of dilution, centrifugation, and swim-up separation on sperm viability and structural morphology in teratospermic domestic cat ejaculates*

Teratospermic ejaculates	Sperm treatment§			
	Undiluted raw	Diluted raw	Non-swim-up	Swim-up
Sperm motility (%)	73.3 ± 1.2*	64.7 ± 1.2†	63.6 ± 1.2†	88.6 ± 1.2‡
Sperm progressive motility	3.7 ± 0.1*	3.3 ± 0.1†	3.4 ± 0.1†	4.0 ± 0.1‡
Sperm motility index	73.6 ± 1.1*	65.6 ± 1.1†	66.3 ± 1.1†	84.0 ± 1.1‡
Normal spermatozoa (%)	33.8 ± 2.3*	22.7 ± 2.3†	25.8 ± 2.3†	56.4 ± 2.3‡
Abnormal spermatozoa (%)				
macrocephalic	0.2 ± 0.04	0.1 ± 0.04	0.1 ± 0.04	0.0
microcephalic	0.2 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.0
bicephalic	2.1 ± 0.2	1.5 ± 0.2	1.6 ± 0.2	0.2 ± 0.2
tricephalic	0.8 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.1 ± 0.1
mitochondrial shealth aplasia	0.4 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
tightly coiled flagellum	5.3 ± 0.8	10.0 ± 0.8	8.7 ± 0.8	2.3 ± 0.8
bent midpiece with droplet	24.1 ± 1.8	14.6 ± 1.8	14.3 ± 1.8	11.5 ± 1.8
bent midpiece without droplet	2.3 ± 0.5	4.8 ± 0.5	4.1 ± 0.5	2.6 ± 0.5
bent flagellum with droplet	5.4 ± 1.5	19.9 ± 1.5	18.9 ± 1.5	7.4 ± 1.5
bent flagellum without droplet	8.2 ± 1.8	21.7 ± 1.8	21.5 ± 1.8	15.9 ± 1.8
proximal cytoplasmic droplet	5.1 ± 0.3	2.3 ± 0.3	2.5 ± 0.3	2.0 ± 0.3
distal cytoplasmic droplet	12.1 ± 1.6	1.8 ± 1.6	1.7 ± 1.6	1.5 ± 1.6

*†‡ Within rows, means with different superscripts differ ($P < 0.05$).
§ Values are means ± pooled SEM of combined media treatment groups (BWW and mKRB).
Each value is based on 18 ejaculates from three normospermic cats (six ejaculates/male).

Compared with the DR and NS treatments, SU processing reduced the percentage of structural abnormalities but the incidence of flagellar bending was not decreased to the extent observed in the UR aliquots (Table 2). In teratospermic cats, the SU treatment improved ($P < 0.05$) spermatozoal progressive motility and increased ($P < 0.05$) the percentage of normal spermatozoa by ~ 1.5-fold over the proportion found in the original, undiluted ejaculate (Table 3). Compared with the UR values, significant reductions were observed in the percentage of spermatozoa with bicephaly, tricephaly, a coiled flagellum, bent midpiece (with droplet), and residual cytoplasmic droplets; the only abnormality increased by SU processing (over the UR treatment) was a doubling in the percentage of spermatozoa with a bent flagellum (without droplet). Compared with the original UR aliquot, SU processing improved ($P < 0.05$) overall sperm motility and motility index in both normospermic (Table 2) and teratospermic (Table 3) cats.

Recovery of total numbers of spermatozoa/ml after swim-up processing was similar ($P > 0.05$) between normospermic (mean spermatozoal concentration, 18.6×10^6) and teratospermic (mean, 17.7×10^6) cats. Inexplicably, the teratospermic group originally produced a higher overall sperm concentration/ml than normospermic counterparts (Table 1). Therefore, although the same processing procedures were used, SU treatment resulted in a much lower ($P < 0.01$) percentage of spermatozoa recovered in the teratospermic (4.9%) compared with the normospermic (11.1%) group.

In normospermic cats, there was no effect ($P > 0.05$) of dilution medium on results. In teratospermic cats, medium influenced seminal traits in certain sperm treatment groups. A greater ($P < 0.05$) mean percent sperm motility (68.3 ± 1.7) and motility index (68.6 ± 1.5) resulted in DR aliquots processed in mKRB compared with BWW processing (61.1 ± 1.7, 62.5 ± 1.5, respectively). Also, a higher ($P < 0.05$) progressive motility (4.2 ± 0.1) and motility index rating (87.8 ± 1.5) were detected in the teratospermic samples following dilution in mKRB and SU processing compared to the SU group diluted in BWW (3.7 ± 0.1, 80.3 ± 1.5, respectively).

Hormonal Assessment

Within each population, mean FSH levels did not vary ($P > 0.05$) between the pre-EE and post-EE blood sample. Mean serum FSH concentrations were 33% lower in teratospermic cats compared with normospermic males but the differences were not significant ($P > 0.05$, Fig. 2). Mean serum LH concentrations also were similar ($P > 0.05$) between normospermic and teratospermic cats and did not vary with the time of sampling (Fig. 2). However, average circulating testosterone concentration was consistently 2- to 3-fold higher ($P < 0.01$) in the normospermic compared with the teratospermic cats (Fig. 2). Testosterone levels decreased ($P < 0.05$) between the pre-EE and post-EE sampling time but only in normospermic males.

Duration of Spermatozoal Motility

Undiluted raw (UR) spermatozoa from each cat population demonstrated similar ($P > 0.05$) sperm motility indices after 6 h incubation (normospermic, 4.6 ± 2.9 versus teratospermic, 3.3 ± 3.3; Fig. 3). Sperm motility was prolonged ($P < 0.05$) by adding culture medium to raw semen, although type of medium (BWW versus mKRB) had no affect ($P > 0.05$). Spermatozoal motility was not affected by centrifugation as demonstrated by similar profiles between the DR and NS groups. For the normospermic group, SU improved the overall 6 h motility index to 85.4 ± 1.9 which was superior ($P < 0.05$) to the UR (4.6 ± 2.9), DR (60.8 ± 2.8) and NS (63.8 ± 3.3) treatments. SU processing resulted in a similar high motility index (80.0 ± 2.2) in the teratospermic males which contrasted ($P < 0.05$) to the values observed after UR (3.3 ± 3.3), DR (53.8 ± 2.0) and NS (55.4 ± 2.0) treatment.

Discussion

The present study determined that domestic cats varied in electroejaculate characteristics and that certain males consistently differ, especially in the prevalence of structurally abnormal spermatozoa produced. Cats ejaculating a high incidence of pleiomorphic spermatozoa also generally produced greater overall sperm concentrations. However, ejaculate volume, percent sperm motility, progressive motility, the number of motile spermatozoa/ejaculate, and testes volume were similar between normospermic and teratospermic males. Of particular interest was the apparent relationship between sperm structural morphology and gonadal androgen production. Although FSH and LH concentrations were similar, teratospermic males consistently produced lower circulating levels of testosterone than normospermic cats. In this context, these data support earlier and similar studies of sperm-endocrine interrelationships in two species of non-

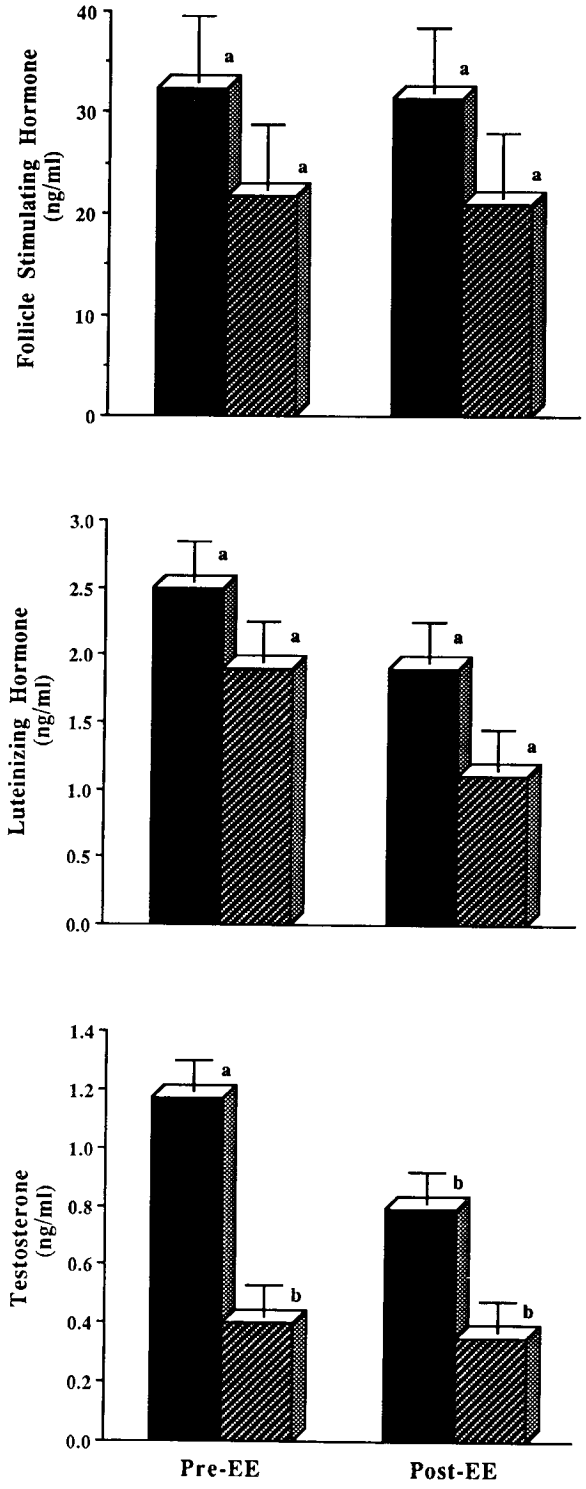

Fig. 2. Mean ± SEM serum FSH, LH and testosterone concentrations in normospermic (solid bars; n = 72 sera samples) and teratospermic (hatched bars; n = 72 sera samples) cats before (Pre-EE) and after (Post-EE) electroejaculation. Within time periods, bars with different superscripts differ significantly ($P < 0.05$).

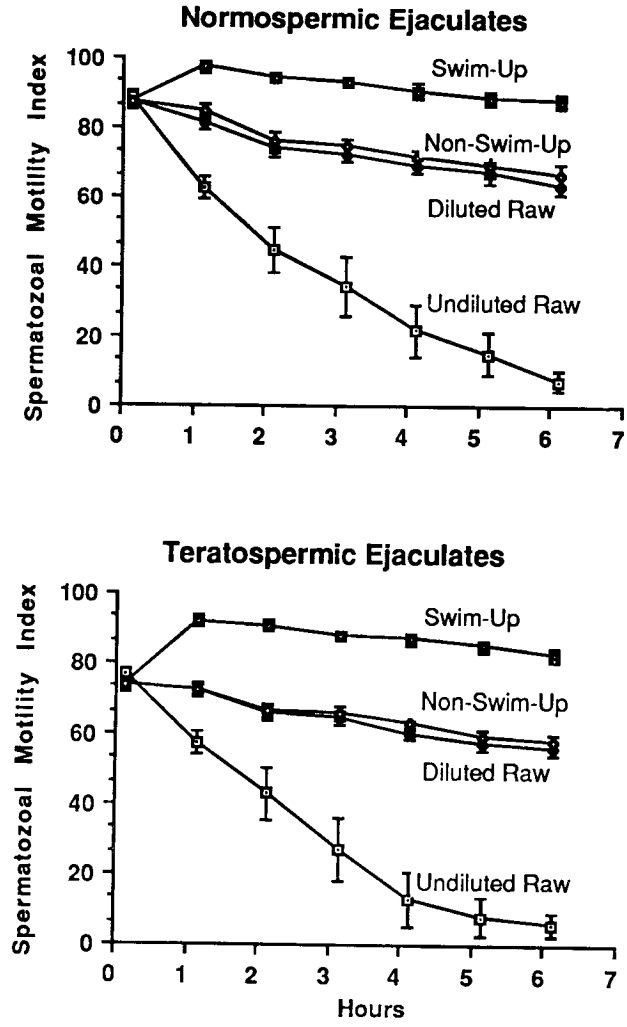

Fig. 3. Influence of semen processing on longevity of sperm viability *in vitro* in normospermic (top panel) and teratospermic (bottom panel) cats. Aliquots of raw semen (undiluted raw) were compared with aliquots diluted in BWW or mKRB medium (diluted raw) or centrifuged for either swim-up sperm separation (swim-up) or resuspension in supernatant (non-swim-up). Within each sperm treatment, there was no difference ($P > 0.05$) in longevity patterns between semen diluted in BWW and mKRB medium so the data were pooled.

domesticated felids. Historically known for poor reproductive performance, the cheetah is genetically monomorphic and produces an unusually high proportion (65–76%) of morphologically abnormal spermatozoa/ejaculate (Wildt et al, 1983, 1987a, 1988). Serum testosterone levels in anesthetized cheetahs generally range from 0.3–0.5 ng/ml (Wildt et al, 1987a, 1988), a similar concentration to that measured in the teratospermic domestic cats of this study. Because all cheetahs have a restricted genotype and produce similar ejaculate character-

istics (Wildt et al, 1987a), it has been difficult to unequivocally associate the lack of genetic variability with compromised sperm integrity and circulating testosterone concentrations. More recent studies have found such evidence by making parallel evaluations of two free-ranging lion populations in Tanzania. One population is genetically variant and produces approximately 25% pleiomorphic spermatozoa in the presence of 1.2–1.8 ng/ml of circulating testosterone (Wildt et al, 1987b). Another population, which is geographically isolated and is known to have experienced a population bottleneck, displays reduced genetic variability, ~ 50% structurally abnormal spermatozoa/ejaculate, and serum testosterone levels of 0.4–0.7 ng/ml (Wildt et al, 1987b). The present observations appear to confirm that there is a causal relationship between gonadal androgen production and pleiomorphic sperm numbers in the domestic cat. This anomaly appears to occur in the presence of normal pituitary function. The literature contains few reports on the relationship of circulating testosterone and incidence of sperm pleiomorphisms in non-carnivore species. However, fertility disorders in stallions exhibiting poor libido have been associated with low basal testosterone levels and ejaculate containing as many as 91% abnormal sperm forms (Bader et al, 1988).

The incidence of abnormal spermatozoa and the specific types of sperm abnormalities detected in the normospermic cats of this study were almost exactly the same as those reported earlier in a similar evaluation of random male cats in this laboratory (Wildt et al, 1983). In the normospermic males in our present study, the most common morphological abnormality was a spermatozoon with a bent midpiece or cytoplasmic droplet. Teratospermic males had a two-fold greater incidence of this specific sperm form and produced more cells with bent or coiled flagella and a variety of head deformities compared with normospermic males. The incidence of specific types of sperm abnormalities appears to be species-specific. The cheetah, leopard, and puma ejaculate contains many spermatozoa with tighly coiled flagella or bent midpieces whereas lion semen contains spermatozoa with a higher incidence of cytoplasmic droplets and flagellar bending (Wildt et al, 1983, 1987b, 1988). In one geographically-isolated subspecies of puma (*Felis concolor coryii*), 50% of all spermatozoa exhibit an abnormal acrosome (Miller et al, 1990). The precise etiology of specific pleiomorphic forms in felids is unknown, but is not attributable to electroejaculation because: cats repeatedly electroejaculated over a relatively short interval continue to produce comparable numbers of sperm pleiomorphisms (Wildt et al, 1983); cheetahs trained to ejaculate into an artificial vagina produce similar numbers of spermatozoal defects as observed in electroejaculates (Durrant et al, 1985); and high numbers of abnormal sperm forms have been recovered from the cauda epididymidis and ductus deferens of selected felid species at necropsy (Howard and Wildt, unpublished).

Sperm pleiomorphisms in fresh, undiluted semen can result from defects in spermatogenesis (Zukerman et al, 1986) or sperm transport through the excurrent duct system (Holt, 1982). In humans, a high proportion of sperm head defects has been attributed to abnormal meiosis (Zukerman et al, 1986). Teratospermic cats had an eight-fold increase in the number of serious sperm head deformities (3.3%, including macro- and microcephaly and bi- and tricephaly) compared with normospermic males (0.4%). Teratospermic cats also produced spermatozoa with an aberrant mitochondrial sheath, similar to the midpiece anomaly known as aplasia or segmental hypoplasia observed in bulls with defective spermatogenesis (Coubrough and Barker, 1964). In boars, Holt (1982) found few abnormal spermatozoa in the caput epididymidis whereas the midpiece of cells recovered distal to the caput were bent and contained a cytoplasmic droplet positioned inside the bend. This particular epididymal anomaly has been reported in several studies (Einarsson and Gustafsson, 1973; Bonte et al, 1978). The most prevalent abnormality observed in teratospermic cats was a bent midpiece with a cytoplasmic droplet positioned within the bend, similar to that described for the boar. The results of previous studies and the observations presented in this report suggest that the incidence of structurally abnormal spermatozoa observed in fresh cat ejaculates was related to both compromised spermatogenesis and epididymal transport. Furthermore, the diminished serum testosterone concentrations observed in the teratospermic cats may be influencing or directly altering normal testicular and epididymal function.

Interestingly, teratospermic cats produced about twice as many spermatozoa/ml of ejaculate as normospermic males. There is a relationship between polyspermia (defined as $> 200 \times 10^6$ spermatozoa/ml of ejaculate) and fertility disorders in humans (Glezerman et al, 1982). A clear mechanism for how polyspermia impairs fertility in humans has not been determined, although reduced

seminal fructose concentrations (Barnea et al, 1980) and a greater incidence of functionally defective acrosomes (Schill and Feifel, 1984) have been suggested as possibilities. The significance of the variation in sperm concentration between the two populations of domestic cats in the present study may have biological relevance. However, these findings also may be inconsequential as there were no differences between groups in the overall number of motile spermatozoa/ejaculate.

Adding tissue culture media to raw ejaculates from either cat population increased pleiomorphic sperm numbers by increasing the proportion of cells with a bent flagellum or midpiece. Osmotic stress and a hypo-osmotic environment have been related to flagellar abnormalities and decreased viability in spermatozoa collected from domestic bulls and men (Lindahl and Drevius, 1964; Makler et al, 1981). A hypo-osmotic solution reduces sperm motility and increases flagellar angulation in these species (Makler et al, 1981), whereas a hypertonic medium improves the opportunity for the capacitation-acrosomal reaction and thus enhances the ovum penetration process (Aitken et al, 1987). We retrospectively analyzed the osmolality of the BWW and mKRB media using a vapor pressure osmometer. Both media had an osmolality of 275–280 mOsm/kg, which is less than cat seminal plasma (323 mOsm/kg, Johnston et al, 1988) suggesting that osmotic stress may be a major cause of increased flagellar bending after tissue culture dilution. Variation in osmolarity can be controlled by adding sodium chloride to either BWW or mKRB during medium preparation which will allow processing spermatozoa in an isotonically and hypertonically defined medium.

Regardless of the adverse effects of tissue culture dilution on flagellar angulation, the swim-up processing procedure negated most of the detrimental effects of dilution alone. The swim-up procedure has been examined previously for its ability to improve a spermatozoal population by separating spermatozoa on the basis of motility (Drevius, 1971; Makler et al, 1984) and structural morphology (Russell and Rogers, 1987). Our results demonstrated that the swim-up approach was very effective for recovering high proportions of motile, structurally normal spermatozoa from male cats prone to producing an extremely high incidence of pleiomorphic spermatozoa. In this context, this processing method appears to be as effective for use in carnivores as previously reported for bulls (Parrish and Foote, 1987) and humans (Jeulin et al, 1986).

Sperm viability in normospermic ejaculates was unaffected by the culture media assessed in this study; however, maximum seminal traits in teratospermic ejaculates were achieved after dilution in mKRB medium. The major difference between BWW and mKRB was a greater concentration of sodium pyruvate and BSA in the latter. It is well-established that sodium pyruvate is an important energy source for sperm motility (Bavister and Yanagimachi, 1977) and that a protein such as albumin facilitates sperm viability and fertilization (Aitken et al, 1983). Perhaps because of lower circulating concentrations of testosterone in teratospermic cats, accessory gland secretions and seminal plasma constituents may be altered resulting in a quantitative and/or qualitative deficiency of proteins and energy substrates in the ejaculates. Therefore, mKRB medium may provide the additional components needed to facilitate sperm viability in teratospermic ejaculates.

Goodrowe et al (1989) recently reported that undiluted cat spermatozoa fail to maintain a high motility index *in vitro* without simple medium dilution that prolongs sperm motility. Our observations confirmed this finding and furthermore determined that incubation environment was important for sustaining sperm motility. In Goodrowe's study, diluted semen was incubated at 37 C in room atmosphere resulting in an 18% spermatozoal motility rating and a spermatozoal motility index of less than 30 at 1.5 h. In the present study, diluted ejaculates incubated at 25 C for 1 h and then transferred to a 5% CO_2 in air, humidified environment at 37 C had motility indices of 63–73 after 2 h of incubation, perhaps as a result of improved buffering causing a stabilized pH.

This study has demonstrated that some domestic cats consistently produce high proportions of structurally abnormal spermatozoa, a finding which may be related to gonadal insufficiency in testosterone production. Simple dilution of teratospermic ejaculates with common tissue culture medium will not improve semen quality and actually will contribute to increased flagellar defects. Swim-up processing permits recovering a high proportion of structurally normal and viable cells from teratospermic ejaculates and, thus, may play an important role in artificially breeding domestic cats. Because *in vitro* fertilization recently has been demonstrated as a successful approach for producing domestic cat offspring (Goodrowe et al, 1988), the ability to

recover viable gametes from teratospermic males with low circulating testosterone concentrations appears especially important. Swim-up sperm recovery also could be extremely valuable to captive breeding programs involving nondomestic felids, many of which are formally classified as endangered (CITES, 1973) and produce a high incidence of sperm pleiomorphisms. Our data affirm that the male domestic cat is a valuable model for studying the etiology and impact of teratospermia in a variety of Felidae species. In this context, studies are in progress to evaluate fertilization potential of spermatozoa collected from normospermic versus teratospermic domestic cats.

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