Seasonal effects on seminal and endocrine traits in the captive snow leopard (*Panthera uncia*)

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The annual reproductive cycle of the male snow leopard (*Panthera uncia*) was characterized by evaluating seminal and endocrine traits monthly. Testicular volume was greatest ($P < 0.05$) during the winter months when the quality of ejaculate was optimal. Ejaculate volume, total sperm concentration ml^{-1} , motile sperm concentration per ejaculate, sperm morphology and sperm motility index were lowest during the summer and autumn months compared with the winter and spring. Peripheral LH, FSH and testosterone concentrations were also lowest during the summer months, increasing during the autumn just before the increase in semen quality, and were maximal during the winter months. There was a direct relationship ($P < 0.01$) between: (1) testosterone and testicular volume, total sperm concentration ml^{-1} , motile sperm concentration per ejaculate and ejaculate volume, and (2) LH and testicular volume and motile sperm concentration per ejaculate. In summary, although spermatozoa were recovered throughout the year, optimal gamete quality was observed during the winter and spring. Although previous studies in felids have demonstrated seasonal effects on either seminal or endocrine traits, this is the first study to demonstrate a distinct effect of season on both pituitary and testicular function.

Introduction

The Felidae represents a unique taxon comprising 37 species, many of which have adapted to a wide array of environmental conditions in the wild. The snow leopard (*Panthera uncia*) inhabits alpine and subalpine areas of central Asia at 5000 m, descending only in the winter to about 1500 m (Jackson, 1991). The species has an enormous range across 12 international boundaries, from the Hindu Kush mountains of Afghanistan to the Himalayan mountains of Nepal and Bhutan. Owing to the isolated and rugged terrain in which it is found, the snow leopard is one of the least-studied large cat species. The snow leopard is considered a highly endangered species, and numbers in the wild have been greatly reduced as a result of: (1) eradication of its prey base; (2) poaching for the fur trade; (3) human encroachment on habitat; and (4) human persecution. Because of the discontinuity of its mountainous habitat, snow leopards do not occupy a continuous range, but rather exist in many subpopulations. Although the prey base of the snow leopard has been increasing in some regions of its range (Smirnov *et al.*, 1990), it is not known whether these subpopulations are viable, or whether sufficient exchange of genetic material can occur between subpopulations without positive human intervention.

Reproductive success is the key to species survival. However, it is well known that when the effective population size is

reduced, genetic diversity is depleted and a cascade of events takes place that causes an immediate loss of fitness and a long-term loss of evolutionary potential and flexibility (Gilpin and Soule, 1986). There are over 200 snow leopards in captivity worldwide, and managed captive breeding programmes have been established in North America and Europe (Blomqvist, 1990). Recently, captive propagation has become an integral component of snow leopard conservation through genetically managed captive breeding programmes using assisted reproductive technology such as artificial insemination and *in vitro* fertilization (Ballou, 1992; Wildt, 1992). However, to make efficient use of assisted reproductive strategies in the management of this species, it is essential that we first understand basic reproductive characteristics including seasonal influences on snow leopard reproductive capacity. The objectives of this study were (1) to examine the annual testicular cycle of the male snow leopard, including seminal and endogenous hormonal characteristics, and (2) to analyse parturition records to determine any potential seasonal effect on the oestrous cycle of the female.

Materials and Methods

Animals

Three adult male snow leopards of prime breeding age (7, 9 and 11 years) were maintained at the Henry Doorly Zoo,

Omaha, NE (latitude 41°, longitude 96°). All males are proven breeders and have sired offspring as recently as 1991. Except during the breeding season, males were housed individually in indoor-outdoor enclosures and exposed to the natural photoperiod throughout the year. A commercial, nondomestic carnivore diet (I & M Industries, Lincoln, NE) was provided 6 days a week.

Electroejaculation, collection of blood samples and semen evaluation

One day each month ($n=12$ evaluations per male), males were anaesthetized ($14.2 \text{ mg ketamine kg}^{-1}$ and $0.5 \text{ mg xylazine kg}^{-1}$) by blow darting, and semen was collected using a standardized electroejaculation technique (Wildt *et al.*, 1983). Briefly, a rectal probe (diameter, 2.5 cm; length, 26 cm) and electrostimulator (AC, 60 Hz current; P-T Electronics, Boring, OR) were used to deliver a regimented electroejaculation sequence consisting of a total of 80 stimuli given in three series (I, II, III). The length and width of each testis was measured and the values were converted to testicular volume (V) using the formula for a prolate sphere ($V = \frac{4}{3}\pi ab^2$, where $a = \frac{1}{2}$ length and $b = \frac{1}{2}$ width; Howard *et al.*, 1986). The volumes for the right and left testes were combined to obtain the total testicular volume per male.

Blood samples (5–10 ml) were collected by saphenous venepuncture immediately before the onset of electroejaculation, immediately after each series of electroejaculations and 15 min after electroejaculation. Samples were centrifuged at 20°C (1200 g, 20 min) 1 h after collection, and the recovered sera stored at –20°C until hormone analysis by radioimmunoassay.

Semen from each series of ejaculations was immediately evaluated for percentage sperm motility and progressive status (at a magnification of $\times 200$); the speed of forward progression was based on a scale of 0 (no movement) to 5 (rapid forward movement) (Wildt *et al.*, 1983). The spermatozoa were then pooled and evaluated for total ejaculate volume, percentage motility and progressive status. Semen was then diluted to 0.5×10^6 motile spermatozoa ml^{-1} in Sperm Washing Medium (SWM; Irvine Scientific, Irvine, CA), maintained at 37°C and evaluated every 2 h for a total of 6 h for motility and progressive status. For each ejaculate, a sperm motility index (SMI) was calculated to provide an overall evaluation of sperm motility characteristics ($\text{SMI} = [\text{sperm \% motility} + (\text{forward progressive motility} \times 20)]/2$) (Howard *et al.*, 1990). An undiluted aliquot of 10 μl of semen was used to determine the sperm concentration in a haemocytometer (Wildt *et al.*, 1983). Sperm morphology evaluations were performed by fixing a 25 μl aliquot in 100 μl of 1% glutaraldehyde and examining 150–200 individual sperm cells using phase contrast microscopy ($\times 1000$) (Wildt *et al.*, 1983). Spermatozoa were classified as normal or having one of the following abnormalities: macrocephalic; microcephalic; bicephalic; malformed head shape; malformed acrosome; mitochondrial sheath aplasia (including segmental or complete aplasia of the mitochondrial sheath); tightly coiled flagellum; biflagellate; bent flagellum; bent neck; bent midpiece with or without cytoplasmic droplet; and a proximal or distal cytoplasmic droplet.

Radioimmunoassays

LH. Serum LH was measured using a heterologous double-antibody radioimmunoassay described by Brown *et al.* (1991a). The assay used a rabbit anti-bovine first antibody (PKC-242; J. L. Brown, Uniformed Services University, Bethesda, MD), an ovine LH label (LER-1374-A; L. E. Riechert, Jr, Albany Medical School, Albany, NY), an ovine LH standard (NIH-LH-S18; NIDDK, National Hormone and Pituitary Program, Rockville, MD) and a sheep anti-rabbit γ -globulin second antibody in a phosphate-based buffer system ($0.01 \text{ mol phosphate l}^{-1}$, $0.14 \text{ mol NaCl l}^{-1}$, $0.002 \text{ mol EDTA l}^{-1}$, 0.5% BSA, pH 7.4). The assay was modified to accommodate a smaller incubation volume (300 μl compared with 1000 μl) and a shorter incubation time (3 days compared with 7 days).

Briefly, serum or standard (100 μl) and first antibody (100 μl ; 1:200 000 final dilution) were added on day 1 and incubated for 24 h at room temperature. On day 2, ^{125}I -labelled LH (100 μl , approximately 20 000 c.p.m.) was added and incubated for an additional 24 h at room temperature. Separation of free from antibody-bound hormone was achieved on day 3 after incubation for 1 h with 1 ml buffer containing second antibody (1:1000 final dilution) and 5% polyethylene glycol (8000 kDa, Sigma Chemical Co., St Louis, MO) and centrifugation at 3000 g for 30 min at 4°C. The LH antiserum bound 25% of the ^{125}I -labelled LH. The standard curve ranged from 0.016 to 4.0 ng per tube with an ED_{50} value of 0.21 ng per tube. Assay sensitivity (determined as 90% of maximum binding) was 0.02 ng per tube (0.2 ng ml^{-1}). The assay was validated for the snow leopard by demonstrating parallelism between dilutions of serum and the LH standard curve. Addition of 0.063, 0.125, 0.25, 0.5, 1 and 2 ng ovine LH to snow leopard serum resulted in a recovery of 101% after subtraction of endogenous hormone ($y = 0.98x + 0.01$; $r = 0.99$). All samples were analysed in a single assay with a 5.6% intra-assay coefficient of variation.

FSH. Serum FSH was measured using a radioimmunoassay (Brown *et al.*, 1987) previously validated for felid serum (Brown *et al.*, 1988, 1991b). The assay used a rabbit anti-ovine FSH first antibody (JAD 178; J. A. Dias, Wadsworth Institute, Albany, NY), an ovine FSH label (LER-1976-A2; L. E. Reichert, Jr, Albany, NY), an ovine FSH standard (NIH-FSH-S8; NIDDK, National Hormone and Pituitary Program) and a sheep anti-rabbit γ -globulin second antibody. The assay was modified as described above for the LH assay. The FSH antiserum bound 30% of the ^{125}I -labelled FSH, and the standard curve ranged from 0.098 to 25.0 ng per tube, with an ED_{50} value of 3.85 ng per tube. Assay sensitivity was 0.25 ng per tube (2.5 ng ml^{-1}). The assay was validated for the snow leopard by demonstrating parallelism between dilutions of serum and the FSH standard curve. Addition of 0.39, 0.78, 1.56, 3.13, 6.25 and 12.5 ng ovine FSH to snow leopard serum resulted in a net recovery of 98% ($y = 1.02x - 0.05$; $r = 0.99$). All samples were analysed in a single assay with a 6.1% intra-assay coefficient of variation.

Testosterone. Serum testosterone was measured using a double-antibody ^{125}I radioimmunoassay kit (ICN Biomedicals, Inc., Costa Mesa, CA). The assay was validated by demonstrating parallelism between dilutions of unextracted snow leopard serum and the testosterone standard curve. Addition of 0.05,

0.125, 0.25, 0.5, 1.25 and 2.5 ng testosterone to snow leopard serum resulted in a net recovery of 103% ($y = 1.03x + 0.08$; $r = 0.99$). The assay sensitivity was 0.05 ng ml^{-1} . All samples were analysed in a single assay with a 5.4% intra-assay coefficient of variation.

Demography Survey data on captive snow leopard females were taken from the International Snow Leopard Studbook (Blomqvist, 1990). Information on the proportion of parturitions for each month of the year was analysed to determine the effect of season on female reproductive patterns.

Statistical analysis The year was divided into four seasons: winter (Dec–Feb), spring (Mar–May), summer (Jun–Aug) and autumn (Sep–Nov). For each animal, mean ($\pm$ SEM) values were calculated for seminal and hormonal characteristics ($n = 5$ observations per male per evaluation) obtained after each ejaculation procedure; the data were then averaged across that season. All data were analysed using a general linear models program (SOLO, BMDP Statistical Software, Inc., Los Angeles, CA). When a significant F value was calculated ($P < 0.05$), differences among means were determined by a least significant difference multiple-comparison procedure. Correlation coefficients were calculated for relationships between the mean values of various hormone concentrations and ejaculate traits.

Results

Female seasonality

Evaluation of 469 snow leopard parturitions within the northern hemisphere demonstrated that births occurred in 7 months of the year, with the greatest number occurring in May (50.3%; 236 of 469) (Fig. 1). Oestrus was observed from January to April, and the duration of gestation was 91–127 days. At the Omaha Zoo, parturitions ($n = 7$) have occurred from March to early August.

Seminal and testis traits

On the basis of a total of 36 collections, the average ejaculate volume was $1.54 \pm 0.1 \text{ ml}$ (range, 0.25–3.2 ml) containing $29.2 \pm 5.7 \times 10^6$ motile spermatozoa ml^{-1} (range, 1.0 – 126.2×10^6) with an average SMI of 76.5 ± 2.4 (range, 43.8–91.3). The mean percentage of morphologically normal spermatozoa was 35.0 ± 2.1 (range, 13.0–55.8%). Within each season, there were no individual differences ($P > 0.05$) in testicular volume, total sperm concentration ml^{-1} , motile sperm concentration per ejaculate, sperm morphology or SMI.

The testicular volume during the winter ($11.4 \pm 1.1 \text{ cm}^3$) was greater ($P < 0.05$) than during the spring ($9.5 \pm 0.5 \text{ cm}^3$), summer ($8.9 \pm 0.5 \text{ cm}^3$) and autumn ($8.8 \pm 0.6 \text{ cm}^3$) (Fig. 2a). Values during the spring, summer and autumn were similar ($P > 0.05$).

During the winter, spring and summer, male 2 consistently produced a greater ($P < 0.05$) ejaculate volume than did males 1 and 3 (Fig. 2b). Analysis of the overall ejaculate volume revealed seasonal differences ($P < 0.05$); higher volumes were produced in the spring ($2.00 \pm 0.2 \text{ ml}$) compared with during

Fig. 1. Percentage of captive snow leopard births per month over the past 15 years.

the summer and autumn ($1.30 \pm 0.1 \text{ ml}$, $1.30 \pm 0.1 \text{ ml}$, respectively). Ejaculate volumes during the winter ($1.79 \pm 0.5 \text{ ml}$) were intermediate and not different ($P > 0.05$) from the other three seasons.

The total sperm concentration ml^{-1} of ejaculate during the winter ($36.3 \pm 7.7 \times 10^6$) and spring ($38.7 \pm 5.3 \times 10^6$) was similar ($P > 0.05$) and greater ($P < 0.05$) than during the summer ($14.2 \pm 3.1 \times 10^6$) and autumn ($6.9 \pm 1.3 \times 10^6$) (Fig. 2c).

The motile sperm concentration per ejaculate during the winter ($65.5 \pm 10.9 \times 10^6$) and spring ($54.0 \pm 0.9 \times 10^6$) was similar ($P > 0.05$), and greater ($P < 0.05$) than during the summer ($8.0 \pm 1.3 \times 10^6$) and autumn ($6.3 \pm 1.1 \times 10^6$) (Fig. 2d).

The overall SMI was higher ($P < 0.05$) during the spring (86.9 ± 1.1) than during the summer and autumn (69.2 ± 0.3 and 72.2 ± 0.2 , respectively), but was similar ($P > 0.05$) to that observed in the winter (77.5 ± 5.5) (Fig. 2e). When the SMI was measured 6 h after collection of the electroejaculate, no differences ($P > 0.05$) were observed among males or seasons (range, 0–57.5; mean, 13.3 ± 3.2).

Overall, the proportion of structurally normal spermatozoa per ejaculate was similar ($P > 0.05$) during the winter and spring, but was greater ($P < 0.05$) than that during the summer (Table 1). Values in the autumn were lower ($P < 0.05$) than in the winter and were similar ($P > 0.05$) to values obtained during the spring and summer. Across all seasons, the most prevalent abnormalities in spermatozoa were malformed acrosomes, malformed head shapes, a coiled flagellum, a bent midpiece with or without a cytoplasmic droplet, and cytoplasmic droplets (Fig. 3). During the summer and autumn, there was a twofold increase ($P > 0.05$) in the incidence of microcephalic forms, a threefold increase ($P < 0.05$) in mitochondrial sheath abnormalities (segmental or complete aplasia) and a 1.5-fold increase ($P > 0.05$) in bent midpieces.

Fig. 2. Mean ($\pm$ SEM) values for ejaculate characteristics of snow leopards on the basis of season: (a) testicular volume; (b) ejaculate volume; (c) total sperm concentration ml^{-1} ; (d) motile sperm concentration per ejaculate; and (e) sperm motility index. The columns represent values combined from all individuals (■), and individual animals: (□), male 1; (▨), male 2; (▩), male 3. Bars with different superscripts are significantly different between seasons ($P < 0.05$).

Circulating LH, FSH and testosterone concentrations

There were no individual differences ($P > 0.05$) in the mean serum LH, FSH or testosterone concentrations within season or among the samples collected before, during and after electroejaculation. Seasonal changes in serum hormone concentrations

for all animals combined and for individual males are shown (Fig. 4).

Mean LH concentrations were greatest ($P < 0.05$) in the winter ($0.78 \pm 0.08 \text{ ng ml}^{-1}$), lowest ($P < 0.05$) in the summer ($0.29 \pm 0.01 \text{ ng ml}^{-1}$) and intermediate in the spring ($0.38 \pm 0.02 \text{ ng ml}^{-1}$) and autumn ($0.46 \pm 0.09 \text{ ng ml}^{-1}$)

Table 1. Structural morphology of snow leopard spermatozoa

Description	Winter (Dec–Feb)	Spring (Mar–May)	Summer (Jun–Aug)	Autumn (Sep–Nov)
Normal	41.3 ± 4.8 ^a	39.4 ± 2.6 ^{a,b}	26.4 ± 2.3 ^c	32.9 ± 2.4 ^{b,c}
Abnormal:				
Macrocephalic	0.1 ± 0.09 ^a	0.8 ± 0.4 ^a	0.3 ± 0.2 ^a	0.0 ^a
Microcephalic	0.8 ± 0.4 ^a	0.7 ± 0.2 ^a	1.6 ± 0.5 ^a	1.3 ± 0.4 ^a
Bicephalic	0.3 ± 0.14 ^a	0.3 ± 0.1 ^a	0.2 ± 0.1 ^a	0.1 ± 0.1 ^a
Malformed head shape	6.3 ± 2.9 ^a	3.8 ± 1.4 ^a	8.2 ± 3.2 ^a	7.5 ± 2.9 ^a
Malformed acrosome	10.9 ± 1.5 ^a	8.3 ± 0.9 ^a	10.3 ± 1.2 ^a	9.1 ± 1.3 ^a
Abnormal mitochondrial sheath	1.4 ± 0.5 ^a	0.8 ± 0.3 ^a	3.1 ± 0.6 ^b	3.7 ± 0.7 ^b
Tightly coiled flagellum	4.3 ± 2.6 ^a	6.3 ± 2.1 ^a	8.5 ± 2.4 ^a	7.6 ± 1.1 ^a
Bent midpiece with droplet	11.2 ± 5.0 ^a	15.5 ± 1.7 ^a	14.0 ± 2.4 ^a	13.6 ± 2.7 ^a
Bent midpiece without droplet	7.9 ± 1.4 ^a	9.3 ± 1.2 ^a	16.6 ± 3.5 ^a	14.4 ± 2.1 ^a
Proximal or distal droplet	4.7 ± 1.1 ^a	6.3 ± 1.0 ^a	4.4 ± 0.7 ^a	4.0 ± 0.9 ^a
Bent flagellum	1.8 ± 0.9 ^a	1.2 ± 0.3 ^a	1.6 ± 0.5 ^a	1.5 ± 0.6 ^a
Biflagellate	0.2 ± 0.1 ^a	0.2 ± 0.7 ^a	0.0 ^a	0.8 ± 0.7 ^a
Bent neck	1.2 ± 0.4 ^a	1.1 ± 0.3 ^a	1.6 ± 0.3 ^a	1.2 ± 0.3 ^a

Values are mean percentages ± SEM.

Values within rows with different superscripts are significantly different ($P < 0.05$).

(Fig. 4a). Concentrations during the spring and autumn were similar ($P > 0.05$).

In contrast to LH, mean FSH concentrations were high in both the autumn ($5.60 \pm 0.18 \text{ ng ml}^{-1}$) and winter ($5.48 \pm 0.26 \text{ ng ml}^{-1}$); no difference ($P > 0.05$) was observed between those two seasons. Mean FSH concentrations in the spring and summer were similar ($3.98 \pm 0.23 \text{ ng ml}^{-1}$ and $3.66 \pm 0.24 \text{ ng ml}^{-1}$, respectively), and were lower ($P < 0.05$) than those measured during the winter and autumn (Fig. 4b).

The seasonal pattern of testosterone secretion was similar to that observed for LH. Overall, the mean testosterone concentrations were highest ($P < 0.05$) in the winter ($1.45 \pm 0.09 \text{ ng ml}^{-1}$) and lowest ($P < 0.05$) in the summer ($0.24 \pm 0.04 \text{ ng ml}^{-1}$) (Fig. 4c). Concentrations observed in the spring and autumn were intermediate and similar ($P > 0.05$; $0.56 \pm 0.07 \text{ ng ml}^{-1}$ and $0.45 \pm 0.12 \text{ ng ml}^{-1}$, respectively).

There were significant ($P < 0.05$) correlations between LH and testosterone ($r = 0.78$), FSH and testosterone ($r = 0.38$) and LH and FSH ($r = 0.51$) concentrations. Significant positive correlations ($P < 0.05$) were also found between testosterone and testicular volume ($r = 0.41$), testosterone and total sperm concentration ml^{-1} ($r = 0.35$), testosterone and motile sperm concentration per ejaculate ($r = 0.58$), testosterone and ejaculate volume ($r = 0.42$), LH and testicular volume ($r = 0.52$), and LH and motile sperm concentration per ejaculate ($r = 0.37$). Within seminal traits, there was a correlation between testicular volume and motile sperm concentration ($r = 0.40$).

Discussion

This is the first study to document a seasonal influence on both ejaculate and reproductive hormone traits in any felid species. The pattern of regression and recrudescence in testicular volume in the snow leopard has been shown to be associated with alterations in spermatogenic capacity, including overall ejaculate volume, sperm concentration and motility, and sperm morphology characteristics. Testicular volume in the snow

leopard was greatest during the winter, coinciding with optimal ejaculate traits and high hormone concentrations.

Although spermic ejaculates were collected during all the seasons, they contained a relatively high proportion of malformed spermatozoa throughout the year. During the winter and spring, snow leopards produced an average of 60% abnormal sperm forms, which is comparable to the value reported by Howard (1991; 58.7%). However, during the summer and autumn, the percentage of abnormal spermatozoa per ejaculate increased to about 70%. Although the aetiology of sperm pleiomorphisms is unknown, increased structural abnormalities may result from disruptions during spermatogenesis (Lincoln, 1981). In this study, the seasonal decline in testosterone may have been related to the parallel rise in ejaculated sperm pleiomorphisms. The causal relationship between increased sperm abnormalities and reduced concentrations of androgens has been documented in both domestic and nondomestic felids. For example, cheetahs, pumas and teratospermic domestic cats secrete comparatively low concentrations of testosterone ($< 0.5 \text{ ng ml}^{-1}$) and ejaculate a high frequency of pleiomorphic spermatozoa ($> 60\%$).

Endocrine data in the snow leopard support the concept that seasonal regression and recrudescence of testicular function is due to changes in pituitary activity and specifically to alterations in LH and FSH secretion. The active phase of spermatogenesis during the winter months is characterized by high concentrations of LH, FSH and testosterone, and increased size of the testes and ejaculate quality. However, the seasonal peak in circulating FSH occurs during the autumn, which is in agreement with studies in other species in which the seasonal serum concentration of FSH increases before the onset of the breeding season and is associated with testicular recrudescence, rather than with maintaining spermatogenic activity (Lincoln, 1981; Soares and Hoffman, 1981; Sanford *et al.*, 1984). This secretory pattern suggests that FSH is probably important in controlling the functional activity of Sertoli cells to regulate spermatogenesis. In addition, FSH may be partly responsible

Fig. 4. Mean ($\pm$ SEM) (a) serum LH, (b) serum FSH and (c) serum testosterone concentrations in snow leopards on the basis of season. The columns represent values combined from all individuals (■), and individual animals: (□), male 1; (▨), male 2; (▩) male 3. Bars with different superscripts are significantly different between seasons ($P < 0.05$).

for enhancing LH-stimulated testosterone secretion by inducing an increase in the concentration of Leydig cell LH receptors (diZerega and Sherins, 1981).

Although LH concentrations began to increase in the autumn, peak values were not reached until the winter. LH modulates the secretory activity of the Leydig cells (diZerega and Sherins, 1981), and the positive correlation observed between LH and testosterone supports the concept that this functional relationship also exists in the snow leopard. Testosterone and FSH control spermatogenesis by acting directly on the seminiferous tubular epithelium (Courot and Ortavant, 1981); it would appear that this also applies to the snow leopard since testosterone secretion is greatest during the winter when reproductive performance is optimal.

On the basis of analysis of parturition records (this study) and patterns of reproductive steroids (Schmidt *et al.*, 1993) in captive snow leopards, oestrous activity occurs between late December and early April. Field observations also concur that free-ranging females exhibit oestrus from January to March (Jackson, 1991). The potential of seasonal influences on oestrous and testicular cycles has also been documented in the clouded leopard (Wildt *et al.*, 1986a, b; Yamada and Durrant, 1989) and Siberian tiger (Seal *et al.*, 1985; Byers *et al.*, 1990).

The clouded leopard is a tropical species found throughout Asia. Analysis of parturition records for captive females (latitude 36–55°N) indicated that although young can be produced throughout the year, most females are in oestrus during autumn and winter; that is, they appear to respond to decreasing daylength (Yamada and Durrant, 1989). In another study, captive clouded leopard males (latitude 36–40°N) exhibited a significant seasonal effect on testosterone secretion, and concentrations were highest in the winter; however, there was no effect of season on LH secretion or ejaculate traits (Wildt *et al.*, 1986a, b).

The Siberian tiger is a temperate species inhabiting broad-leaved coniferous forests in eastern Russia and northeastern China. Endocrine analysis of three females in captivity (latitude 45°N) revealed that peak oestrous activity occurs from late January to early June (Seal *et al.*, 1985). However, unlike the clouded leopard, the Siberian tiger exhibits an anoestrous period of up to 8 months. In a study of five male Siberian tigers, the highest testosterone concentrations were observed during the autumn and winter, but there was no effect of season on ejaculate quality (Byers *et al.*, 1990). Thus, it would appear that seasonal effects on reproduction in the snow leopard are more conspicuous than in the Siberian tiger, and reproductive hormone concentrations positively correlate with changes in both size of the testes and quality of the ejaculate.

The study reported here demonstrates that optimal reproductive performance in the snow leopard is synchronized between the sexes and is seasonally mediated. There are a variety of environmental elements having the potential to affect seasonal reproduction including dietary (availability and source of food), physical (temperature or rainfall) and social factors. Studies on domestic cats showed that queens are

Fig. 3. Sperm forms detected in the snow leopard ejaculate: (a) normal; (b) coiled flagellum; (c) microcephalic defect and complete aplasia of mitochondrial sheath; (d) abnormal acrosome; (e) bent midpiece with cytoplasmic droplet; (f) segmental aplasia of mitochondrial sheath; (g) bicephalic; and (h) proximal droplet with segmental aplasia of mitochondrial sheath.

seasonally polyoestrus, with periods of anoestrus that are dependent on photoperiod and latitude (Scott and Lloyd-Jacob, 1959; Scott, 1970). Free-ranging queens in the northern hemisphere exhibit oestrus as early as January or February in response to increasing daylength (Herron, 1977; Stabenfeldt and Shille, 1977). However, when maintained under controlled conditions (a photoperiod of 12 h light:12 h dark), queens can exhibit oestrous cyclicity throughout the year (Jemmett and Evans, 1977; Wildt *et al.*, 1978). That photoperiod mediates seasonal reproduction in another felid, the tiger, is suggested by the observation that the duration of anoestrus was shortened in one female exposed to a longer period of daylight. The data reported here also suggest that photoperiod is a possible environmental mediator of both oestrous and testicular activity in the snow leopard.

The goal of any conservation-oriented captive propagation programme is to maintain genetic diversity in a stable population (Foose *et al.*, 1986). Genome resource banks for gametes and embryos are potentially valuable tools in the management of captive species, since they can be used to help maintain the original genetic diversity of the population by extending the lifespan of individuals (Johnston and Lacy, 1991; Ballou, 1992; Wildt, 1992). The Species Survival Plan for the snow leopard, under the auspices of the American Zoo and Aquarium Association, has begun to establish a cryopreservation bank containing spermatozoa of genetically valuable, captive males that is destined for artificial insemination or *in vitro* fertilization. Data about ejaculates from this study will provide useful information for developing gamete collection strategies for the snow leopard.

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