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INFLUENCE OF SEASONALITY ON REPRODUCTIVE TRAITS OF THE MALE PALLAS' CAT (*FELIS MANUL*) AND IMPLICATIONS FOR CAPTIVE MANAGEMENT

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Abstract: A male Pallas' cat (*Felis manul*) was housed under natural lighting conditions in an outdoor pen for 20 mo and subjected to bimonthly reproductive evaluations consisting of blood sampling for hormonal analysis, testicular volume and body weight measurement, and electroejaculation. Distinct seasonal reproductive patterns were identified; sperm production and quality in the breeding season (December–April) was significantly higher ($P < 0.01$) than that in the nonbreeding season (June–October). Testicular volume did not differ ($P > 0.05$) between seasons, but body weight gain and loss occurred ($P < 0.01$) 1–2 mo before the breeding and nonbreeding seasons, respectively. Although serum testosterone concentrations were similar ($P > 0.05$) in both seasons, serum luteinizing hormone (LH) concentrations were greater ($P < 0.01$) during the breeding season, and sperm concentration, total number of sperm per ejaculate, percentage of morphologically normal sperm, sperm motility, and serum LH concentration were correlated ($r = 0.60–0.99$; $P < 0.05$). The male Pallas' cat exhibits a pronounced reproductive seasonality, which has important implications for captive breeding management and genome resource banking.

Key words: Pallas' cat, *Felis manul,* seasonality, reproduction, captive management.

INTRODUCTION

The Pallas' cat (*Felis manul*) is a small (2.5–5.0 kg) felid endemic to the rocky steppes of Siberia and Mongolia and adapted to surviving the frequently harsh winter conditions typical of its native range.[13,17] Wild Pallas' cat populations are threatened due to habitat loss, hunting pressures, and an uncertain political environment in many host countries.[4] Accordingly, this species has been listed in Appendix I of the Convention on the International Trade of Endangered Species of Flora and Fauna (1984 Endangered Species Act, part 23) and designated as a priority small cat species for conservation efforts by the Felid Taxon Advisory Group (TAG) of the American Zoo and Aquarium Association (AZA).[23] The known captive population of Pallas' cats is extremely small (~20 individuals) and geographically dispersed, with a history of low fecundity and a high degree of suspected inbreeding.[4,12] Because our knowledge of reproductive processes in this species is extremely limited, improvements in husbandry and captive breeding management have been hindered. Therefore, the objectives of this study were to 1) characterize basic reproductive traits, especially semen quality, sperm production, and hormonal concentrations, in the male Pallas' cat and 2) assess the impact of seasonality on these characteristics.

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MATERIALS AND METHODS

A male Pallas' cat (estimated age, 8–10 yr) was maintained for 20 consecutive months (June 1993–February 1995) in an outdoor enclosure at the Conservation and Research Center, Front Royal, Virginia (39°N). The cat's enclosure consisted of two interconnected corncrib pens (6 m diameter), each containing a partitioned den box (~1 m^3). The cat's daily diet consisted of Nebraska Canine Diet (Central Nebraska Packing, North Platte, Nebraska 69103, USA) supplemented with a mouse each day and two chicks each week. The cat had been caught in Mongolia in 1985 and was a proven breeder in captivity, previously siring one kitten in 1991, which was cannibalized by the dam. During the first 6 mo

of this study, this cat was housed within visual and olfactory range of a female Pallas' cat but was physically paired with the female only for a brief period (~3 wk) in the winter of 1994. The female died of a respiratory infection shortly after pairing, and no offspring were produced.

Reproductive evaluations of the male cat were conducted bimonthly using a standardized protocol consisting of blood sampling, testicular measurements, and semen collection via electroejaculation.[6,19] Food was withheld for 12–24 hr before each anesthetic episode. Anesthesia was induced with ketamine hydrochloride (Ketaset, Fort Dodge Laboratories, Forth Dodge, Iowa 50501, USA; 20 mg/kg body mass, i.m.) delivered by hand syringe. Prior to electroejaculation, body weight was determined, a blood sample (~7 ml) was collected via jugular venipuncture, and calipers were used to measure dimensions (width, length) of both testes for calculating testicular volume.[6] A rectal probe (1 cm diameter) and an electrostimulator (P. T. Electronics, Boring, Oregon 97009, USA) were used to deliver a structured electroejaculation regimen consisting of two series of 30 electrical stimuli and a third series of 20 stimuli, with incremental increases in voltage (range, 3–5 V, 10–75 mA) and 5–10 min intervals between each series. Recovered semen initially was evaluated for pH, volume, and the presence or absence of spermatozoa. Sperm samples were assessed for percent sperm motility and rate of progressive forward movement (scale of 0–5; 0 = nonmotile, and 5 = rapid forward progression). With this information, a sperm motility index (SMI) was calculated using the formula: (% sperm motility + [20 × rate of progressive movement])/2.[6] An aliquot (5–10 μl) of raw semen was fixed in 100 μl of 0.3% glutaraldehyde for later morphologic examination (200 sperm/ejaculate) using phase contrast microscopy (1,000×), and sperm concentration was determined with 5–10 μl of semen using a red blood cell determination kit (hemacytometer method).[6] The remaining raw semen was diluted 1:1 with Ham's F10 medium (Irvine Scientific, Santa Ana, California 92705, USA) supplemented with 5% fetal calf serum, pyruvate (0.011 mg/ml), penicillin (100 U/ml), and streptomycin (100 μg /ml).

Because this male was wild caught, had no surviving offspring, and represented a potential founder for the captive Pallas' cat population, cryopreservation of recovered sperm was considered a high priority. High-quality semen samples ($\geq 5 \times 10^6$ total motile spermatozoa, ≥70 SMI) were centrifuged (200 *g*, 10 min), the supernatant was discarded, and the resulting sperm pellet was resuspended in 100–200 μl of a cryoprotectant diluent.[14] Extended spermatozoa were cooled at 5°C for 30 min and cryopreserved by pelleting onto dry ice or freezing in straws at a controlled rate over liquid nitrogen vapor.[6,24] Frozen sperm samples were stored in liquid nitrogen as a component of a felid genome resource bank.[18]

Blood samples were centrifuged and sera stored at −80°C until analyzed for testosterone and luteinizing hormone (LH) concentrations. Testosterone concentration was determined using a double antibody ^{125}I radioimmunoassay kit (ICN Biomedicals, Costa Mesa, California 32625, USA), and LH concentrations were assessed using a heterologous double antibody radioimmunoassay. Each radioimmunoassay had been validated previously for felid sera.[2,9] Assay sensitivity was 0.05 ng/ml for both testosterone and LH assays. All serum samples were evaluated in single assays for the respective hormones, with intra-assay coefficients of variation of <10%.

For data analysis, the year was divided into breeding (December–April) and nonbreeding (June–October) seasons based on limited parturition information for female Pallas' cats.[12,15,17] For each season, mean (±SEM) values for body weight, testicular volume, sperm concentration, total sperm/ejaculate, percentage of normal sperm forms, SMI, and serum testosterone and LH

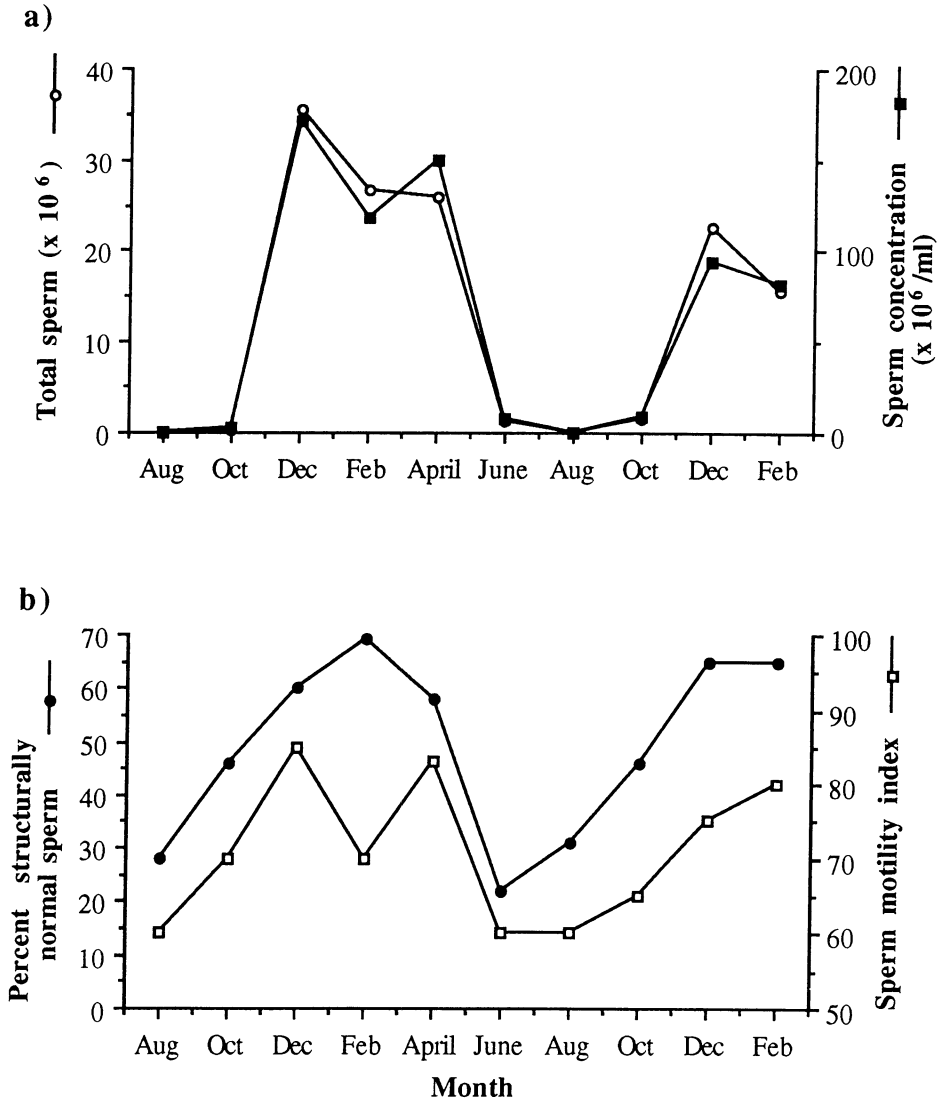

Figure 1. Seasonal variation in semen characteristics in a male Pallas' cat. **a.** Total number of sperm ($\times 10^6$) per ejaculate and sperm concentration ($\times 10^6$) per ml of semen. **b.** Percentage of normal sperm forms and sperm motility index (SMI = [% motility + (20 × forward progressive movement)]/2).

concentrations were calculated, and differences between seasons were analyzed using a Student's *t*-test.[16] Correlation coefficients between reproductive traits were also calculated.[16]

RESULTS

The male cat exhibited seasonal variations in total sperm per ejaculate, sperm concentration, percentage of structurally normal sperm, and sperm motility index with nadirs from June through October and peaks from December through April (Fig. 1a, b). Body weight, testicular volume, and serum testosterone and LH concentrations also exhibited seasonal fluctuations (Fig. 2a, b). Near the onset of the breeding season (September–December), this cat's behavior also became more physically aggressive and territorial, characterized by charging the animal keepers whenever they entered the enclosure. During the breeding season, this cat produced a more concentrated ejaculate ($P < 0.01$) containing more total spermatozoa ($P < 0.01$) than observed in the nonbreeding season (Table 1). Semen contained a higher percentage of normal sperm forms ($P < 0.01$) and greater sperm motility ($P < 0.01$) (Table 1). The higher percentage of malformed sperm observed

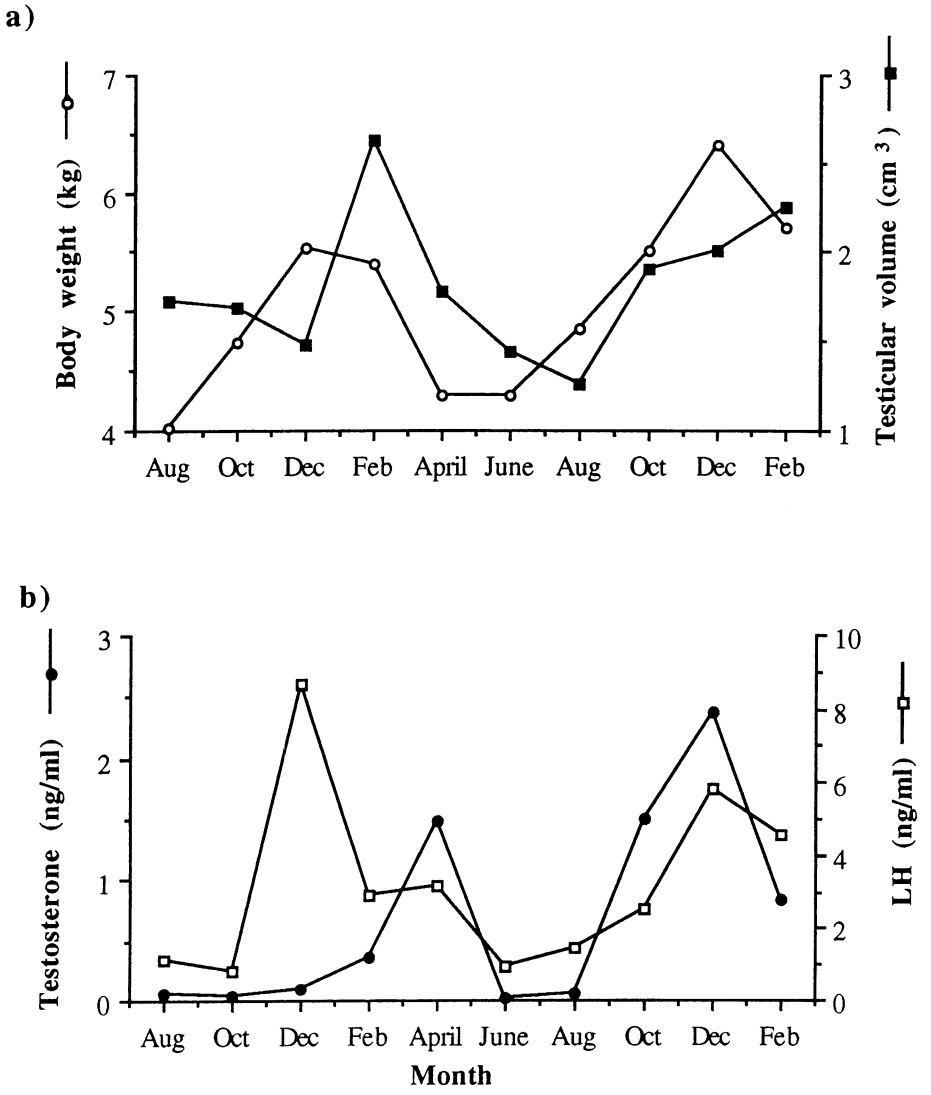

Figure 2. Seasonal variation in morphological and hormonal traits in a male Pallas' cat. **a.** Body weight (kg) and testicular volume (cm^3). **b.** Serum testosterone (ng/ml) and luteinizing hormone (LH) (ng/ml) concentration.

Table 1. Mean (±SEM) values for semen characteristics, testicular volume, body weight, and reproductive hormones during the breeding and nonbreeding season[a] in a male Pallas' cat.

Parameter	Breeding season	Nonbreeding season
Semen volume (μl)	210 ± 10	190 ± 10
Sperm concentration ($\times 10^6$/ml)	123.0 ± 16.7[c]	3.8 ± 1.8[d]
Total sperm/ejaculate ($\times 10^6$)	25.2 ± 3.2[c]	0.7 ± 0.3[d]
Percentage structurally normal sperm forms	63.4 ± 2.0[c]	34.6 ± 4.9[d]
Sperm motility index[b]	78.5 ± 2.7[c]	64.0 ± 1.9[d]
Combined testicular volume (cm^3)	2.0 ± 0.2	1.6 ± 0.1
Body weight (kg)	5.47 ± 0.34	4.68 ± 0.25
Serum testosterone (ng/ml)	1.27 ± 0.41	0.33 ± 0.29
Serum LH (ng/ml)	5.02 ± 1.05[c]	1.37 ± 0.30[d]

[a] Breeding season = December–April; nonbreeding season = June–October.
[b] SMI = [% motility + (20 × rate of forward progression)]/2.
[c,d] Within traits, values with different superscripts differ ($P < 0.01$).

during the nonbreeding season was primarily due to more sperm with abnormal midpieces and proximal droplets. Although serum testosterone concentrations did not differ significantly between breeding and nonbreeding seasons ($P > 0.05$), the cat averaged at least threefold more testosterone during the former period. Serum LH concentrations were greater in the breeding season ($P < 0.01$) (Table 1). Sperm concentration, total sperm per ejaculate, SMI, and percentage of normal sperm forms were correlated with each other ($r = 0.75$–0.99; $P < 0.01$) and with serum LH ($r = 0.60$–0.70, $P < 0.05$). However, LH and testosterone concentrations were not correlated ($r = 0.26$, $P > 0.05$).

Body weight did not differ between defined seasons ($P > 0.05$) (Table 1), but the temporal pattern suggested distinct circannual variation 1–2 mo out of phase with changes in other reproductive characteristics. After adjusting for this asynchrony, body weight for October–February (5.54 ± 0.22 kg) differed significantly ($P < 0.01$) from that in April–August (4.37 ± 0.17 kg). In the second year of the study, the bimonthly schedule of reproductive evaluations was altered slightly by conducting one additional evaluation during the first week of November. Values for reproductive parameters were essentially unchanged from those observed in October but were substantially less than values reported 1 mo later (December) (data not shown).

When sperm quality was acceptable, ejaculates were cryopreserved. For the five reproductive evaluations conducted during the breeding season, 126×10^6 motile spermatozoa of high quality (≥60% normal sperm morphology, ≥70 SMI) were recovered, and all of these spermatozoa were cryopreserved and stored as part of the genome resource bank. Only 3.3×10^6 motile spermatozoa of poor quality (≤50% normal sperm morphology; ≤70 SMI) were collected over the five reproductive evaluations conducted in the nonbreeding season; these spermatozoa were considered unacceptable for cryopreservation.

DISCUSSION

These data represent the first objective evidence that the male Pallas' cat exhibits pronounced reproductive seasonality, perhaps more distinctive than that reported for males of most other felid species. Because only a single male was available for study from the small global captive population, reproductive evaluations were conducted for 18 mo to confirm repeatability of observed seasonal variations and to permit statistical analysis of data. Anecdotal observations indicate that Pallas' cat births in captivity and the wild are concentrated predominately in the months of April and May but have occurred as late as August in zoos.[12,15,17] Based on a gestation length of 74–75 days, this parturition period corresponds to a 2-mo female breeding season (January–February).[15] This short breeding period has been confirmed by longitudinal fecal hormone analysis of female Pallas' cats, which indicated peak values in fecal estradiol metabolites during these 2 mo, with basal levels observed during the remainder of the year (J. L. Brown, unpubl. data). Based on measured reproductive parameters, the breeding season for the male Pallas' cat appears to be more protracted than that of the female, a phenomenon probably related to the lower energy cost of male reproduction.[1]

In both study years, the values for the male reproductive parameters increased in October–December, with an abrupt transition occurring between late November and early December. Reproductive peaks were observed for most traits between December and February, corresponding to the females' reported breeding season, with values returning to baseline between April and June. Similar temporal reproductive patterns were observed during each year, irrespective of the presence or absence of the female Pallas' cat. Analysis of serial electroejaculations revealed that spermatogen-

esis essentially ceased during the nonbreeding season. This reproductive seasonality was more marked than that reported for the domestic cat, tiger (*Panthera tigris*), and clouded leopard (*Neofelis nebulosa*), felid species that display slight seasonal variations in testosterone production and/or testicular size but rarely in seminal traits.[3,11,21,22] The Pallas' cat appears most similar to the snow leopard (*Panthera uncia*), a partially sympatric species subjected to comparable climatic extremes within its natural habitat.[9] Male snow leopards and the Pallas' cat exhibit nearly identical seasonal patterns for seminal characteristics and reproductive hormones, a finding that suggests evolutionary convergence of seasonality in these two distantly related cat species.[9]

Measures of sperm production and quality in the Pallas' cat were highly correlated, but values for body weight and testicular volume generally were not clearly related to other parameters. Despite the constant availability of a palatable diet, the male exhibited temporal changes in body weight, with weight gain and loss typically occurring 1–2 mo before the defined breeding and nonbreeding seasons, respectively. This seasonal fluctuation in body mass probably represents a physiological adaptation for enhancing reproductive fitness during the breeding season and for withstanding the climatic stress of the winter months. In contrast, testicular volume did not exhibit a consistent seasonal pattern, although maximal volume usually corresponded to peak values for other reproductive traits. Circulating hormone concentrations also varied considerably throughout the year. Although serum testosterone generally was elevated between October and April, distinct fluctuations occurred between successive sampling periods. Because LH and testosterone secretion probably is pulsatile in felids, a one-time blood sampling protocol may be insufficient to provide accurate baseline values.[5,10,21] Fecal steroid concentrations, however, are less sensitive to episodic secretory activity, and hormonal metabolite analysis has indicated the occurrence of distinct seasonal changes in fecal testosterone production in male Pallas' cats (J. L. Brown, unpubl.). Serum LH concentrations differed between seasons and were correlated to most other reproductive parameters except serum testosterone. This lack of correlation between LH and testosterone may be the result of the episodic secretory nature of these hormones, the infrequent sampling, and the natural lag time between an LH surge and a subsequent increase in circulating testosterone.

One consequence of the pronounced seasonality in sperm production in the Pallas' cat was the limited availability of quality spermatozoa for cryopreservation during some months. Because of low sperm concentrations and poor sperm quality, all semen samples collected between June and October were unsuitable for cryopreservation, demonstrating the potential impact of seasonality on planning and implementing genome resource banking strategies.[18] Similarly, reproductive seasonality may affect the application of natural breeding and assisted reproduction for captive management. Because males produce high quality semen for ~5 mo each year, the breeding period in Pallas' cats is apparently regulated by the more restricted seasonality of the female. The control mechanisms of seasonality have not been investigated in this species, but photoperiod and ambient temperature should be considered as contributory factors.[7,8,20] For small felid species that can be housed in indoor enclosures, these variables might be easily controlled and manipulated. For example, artificial lighting schedules (i.e., 12 hr light : 12 h dark) are known to stimulate ovarian cyclicity throughout the year in the domestic cat.[7,20] In the Pallas' cat, similar lighting regimens might be useful to promote circannual sperm production, ovarian cyclicity, and breeding, thus reducing the dominant influence of seasonality on the captive management of this threatened species.

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