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Seasonal variation in the endocrine–testicular function of captive jaguars (*Panthera onca*)

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Abstract

Captive adult male jaguars (*Panthera onca*) from two locations in southeast Brazil were studied to evaluate the effects of season on endocrine and testicular function. For assessment of testicular steroidogenic activity, androgen metabolite concentrations were measured in fecal samples collected one to three times per week over 14 ($n = 4$), 9 ($n = 1$) or 7 months ($n = 1$). To assess seasonality, data were grouped by season (summer: December–February; autumn: March–May; winter: June–August; spring: September–November). Additionally, samples collected in the dry season (March–August) were compared with those collected in the wet season (September–February). There were no differences ($P > 0.05$) in fecal androgen concentrations in samples collected in spring, summer, autumn, and winter (480.8 ± 50.4 ng/g, 486.4 ± 42.0 ng/g, 335.4 ± 37.7 ng/g, and 418.6 ± 40.4 ng/g dry feces). However, there were differences ($P < 0.05$) in fecal androgen concentrations between the dry and wet seasons (380.5 ± 28.0 ng/g versus 483.9 ± 32.3 ng/g dry feces). Sperm samples, collected from all males twice (approximately 6 months apart) were similar; mean (±S.E.M.) motility, concentration and morphology were $57.0 \pm 4.5\%$, $6.3 \pm 2.4 \times 10^6$ ml^{-1}, and $60.8 \pm 3.1\%$, respectively. In conclusion, androgen metabolite concentrations in the captive male jaguar were not affected by season, but there was a difference between the wet and dry periods. Further research is needed to verify these results.

Keywords: Fecal androgens; Seasonality; Jaguar; Captive; Semen

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1. Introduction

The jaguar (*Panthera onca*), the only member of the genus *Panthera* in the Americas, is known to have been extirpated in 37% of its original range [1]. Due to deforestation and depletion of prey base as a result of human development, jaguar populations have declined rapidly and are currently listed as endangered by the Brazilian Institute of Environmental Resources and Appendix I of CITES [2]. In addition, populations in the Atlantic tropical moist lowland forest and Brazilian Savannah are at critical levels, with a low probability for long-term survival [3,4].

Jaguars have reproduced poorly in Brazilian zoos over the last 10 years [5]. While the cause of this low fertility is unknown, the increasing age of the population makes immediate and effective management a critical step in improving captive propagation. Strategies, such as captive breeding and assisted reproductive techniques (ART) such as artificial insemination (AI), in vitro fertilization (IVF) and embryo transfer (ET), have been recognized as important tools in assisting genetic management of this species [6,7]. However, the lack of information on reproductive physiology of jaguars has hampered efforts to improve reproduction and to establish genetic exchange among both captive and free-living populations using ART. Fundamental knowledge of basic reproductive physiology is essential to plan and implement genome resource banking strategies that should be used in conjunction with ART [8].

Sub-fertility of captive males may be one reason for the low success rate of natural and assisted breeding (IVF [9]) in captivity. While basic information on the reproductive characteristics of male jaguars has been described [10,11], hormone profiles were generated with a limited number of serum samples [12]. The value of fecal steroid monitoring has been demonstrated for characterizing reproductive events in felids [8,13], including the identification of fluctuations in seasonal reproductive activity of males [8].

The primary objective of this study was to determine the influence of season on reproductive function in male jaguars by monitoring fecal androgen metabolite excretion.

2. Materials and methods

2.1. Animals and fecal sample collection

Male adult jaguars, all captive-born (except for Animal E), were maintained in individual cages with indirect olfactory or visual contact at São Paulo Zoo—Brazil (A, B, and C) or paired with a male (D and E) or female (F) at the Sorocaba Zoo—Brazil. All animals were housed in outdoor enclosures with exposure to natural light and temperature fluctuations. Ages ranged from 3 to 10 years, based on estimated ($n = 1$) or known dates of birth ($n = 5$). During the study, animals at both facilities were fed a meat-based diet, supplemented with a powdered vitamin and mineral mixture to meet the minimum nutrient requirements for domestic cats [14]. All animals were fed once daily, with 1 day fasting each week.

Fecal samples for hormonal analysis were collected from each male 1–3 days per week, placed into cryo-vials, immediately frozen and stored at -196 °C until extraction for fecal hormone analysis. Fecal samples of four of the six animals were collected throughout the study (14 months). Feces were collected from Animal B until it died (7 months). Finally, only 9 months of fecal collection was possible with Animal D, due to management considerations and transfer to another institution after 1 year.

2.2. Semen collection and evaluation

All animals were chemically restrained (tiletamine-zolazepam 10 mg/kg, Zoletil®, Virbac do Brazil, São Paulo, Brazil) once before onset of the study (January 1998) and 6 months later (July 1998) for clinical evaluation and semen collection by electroejaculation, as previously described [11]. Animal F was vasectomized in 1989 but had normal spermatogenesis as confirmed by ultrasonography and fine needle aspiration, as previously described [15].

2.3. Steroid extraction and radioimmunoassay

Fecal samples were processed and analyzed for androgens, as previously described [8,13]. Samples were dried with a Savant Instruments Speedvac Rotary Evaporator (Forma Scientific Inc., OH, USA), pulverized, and 0.2 g was boiled in 5 ml 90% ethanol for 20 min. After centrifugation at $500 \times g$ for 10 min, the supernatant was recovered and the pellet resuspended in 5 ml 90% ethanol, vortexed for 1 min, and recentrifuged at $500 \times g$ for 10 min. Ethanol supernatants from each sample were combined and dehydrated, dissolved in 1 ml methanol, and diluted in gelatin buffer (1:10–1:40; $NaPO_4$ 0.06 M, NaCl 0.4 M and gelatin 0.15 M) in preparation for analysis. The radioimmunoassay was validated by demonstrating: (1) parallelism between binding inhibition curves of fecal extract dilutions and the appropriate steroid standard ($y = 1.3 - 0.92x$; $r^2 = 0.99$); and (2) recovery of more than 80% of exogenous steroid added to fecal extracts.

The androgen assay was performed using a commercial solid phase testosterone kit (Coat-A-Count Total Testosterone, Diagnostic Products Corporation, Los Angeles, CA, USA), previously validated for felids [8]. The following cross-reactivities were provided by the company for the antiserum: 100% testosterone, 34% dihydrotestosterone, 3.8% 5β-androstan-3α,17β-diol, 3.3% 11-hydroxitestosterone, 2.9% 5α-androsten-3α,17β-diol, 2.7% 5α-androsten-3β,17β-diol, 2.1% androsterone, and <1% with androstenediol, DHEA, 5β-dihydrotestosterone, aldosterone, cortisol, cortisone, corticosterone, estradiol, pregnanediol, progesterone, and 17α-hydroxiprogesterone. The minimum detectable dose, based on 91% of maximum binding, was 8 pg per tube. Intra- and inter-assay coefficients of variation for the assays were <10%. All hormonal data are expressed on a dry weight basis.

2.4. Environmental data

Daily records from the Instituto Astronômico de São Paulo and Instituto Agronômico de Campinas were used to assess environmental factors. Monthly means for daily means of environmental temperature (°C) and photoperiod (h) were calculated for the São Paulo Zoo and the Sorocaba Zoo in 1998 and 1999, and correlated with reproductive data.

2.5. Statistical analysis

Values for each individual were averaged on a monthly basis (mean ± S.E.M.), and compared using the Mann–Whitney rank test to assess differences between zoos. To assess seasonality, monthly individual means were grouped by seasons (summer: December–February; autumn: March–May; winter: June–August; spring: September–November) and differences determined by Kruskal–Wallis one-way ANOVA on ranks test. Additionally, samples collected in the dry season (March–August) were compared with those collected in the wet season (September–February) using the Mann–Whitney rank test. Spearman's correlation analysis was performed to evaluate the relationship between fecal androgen metabolites, semen traits, testicular volume, body weight and environmental data. All analyses were carried out using the software program Minitab (Minitab Inc., Version 13.0, 2000).

3. Results

3.1. Environment

Mean monthly values for daily environmental temperature and photoperiod in both locations are shown in Table 1. The two locations did not differ ($P > 0.05$) in mean photoperiod length. However, the photoperiod in summer averaged 2.5 h longer than in winter ($P < 0.05$). Environmental temperature also varied among seasons for both locations ($P < 0.05$), with overall mean daily values being higher ($P < 0.05$) throughout the experimental period in Sorocaba (21.6 ± 0.2 °C) than at São Paulo (19.3 ± 0.2 °C).

3.2. Seminal traits

Electroejaculation elicited full erections in all males at each collection. Data for body weight, testicular volume and semen traits are shown in Table 2. There was no difference ($P > 0.05$) between the first and second collections in any seminal trait, including volume, concentration, motility, forward progressive motility, and morphology.

Table 1
Seasonal mean for daily environmental temperature (°C) and photoperiod (h) in Sorocaba and São Paulo from January 1998 to February 1999

Season	Temperature (°C)[a]		Photoperiod (h)	
	São Paulo	Sorocaba	São Paulo	Sorocaba
Summer (December–February)	22.7 ± 0.1^{a}	25.4 ± 0.1^{a}	13.2 ± 0.1^{a}	13.2 ± 0.1^{a}
Autumn (March–May)	19.6 ± 0.2^{b}	21.6 ± 0.1^{b}	11.4 ± 0.3^{bc}	11.4 ± 0.3^{bc}
Winter (June–August)	16.2 ± 0.3^{c}	17.5 ± 0.2^{c}	10.8 ± 0.1^{c}	10.8 ± 10.1^{c}
Spring (September–November)	18.7 ± 0.4^{b}	22.1 ± 0.1^{b}	12.5 ± 0.3^{ab}	12.5 ± 0.3^{ab}

Values with different superscripts (a, b, c) within columns differ among seasons ($P < 0.05$).
[a] There was a difference in the temperature between the two locations ($P < 0.05$).

Table 2
Mean (±S.E.M.) body weight, testicular volume and semen traits in male jaguars ($n = 5$)

	$n = 10$[a]
Body weight (kg)	70.5 ± 4.5
Testicular volume (cm^3)	44.4 ± 2.0
Semen volume (ml)	6.60 ± 1.9
Sperm concentration ($\times 10^6$)	6.30 ± 2.4
Sperm motility (%)	57.0 ± 4.5
Sperm progressive motility (0–5)	2.80 ± 0.2
Sperm motility index[b]	56.5 ± 4.5
Normal sperm	60.8 ± 3.1

[a] Number of ejaculates.
[b] Sperm motility index: [%motility + (20 × sperm progressive motility)]/2.

3.3. Fecal androgen concentrations

Longitudinal profiles of excreted androgen metabolites in dry feces for individual jaguars A, B, C, D, E and F are represented in Fig. 1. There was a considerable variation among individual fecal androgen concentrations (range: 10.5–1974.0 ng/g dry feces; Table 3), with an overall mean of 429.8 ± 21.3 ng/g dry feces ($n = 455$).

The mean fecal androgen concentration of animals housed at São Paulo Zoo (689.6 ± 31.2 ng/g dry feces) was three times higher ($P < 0.05$) than that of Sorocaba Zoo animals (209.2 ± 20.7 ng/g dry feces).

There was no difference ($P > 0.05$) between fecal androgen concentration during summer, autumn, winter and spring (486.4 ± 42.0 ng/g, 335.4 ± 37.7 ng/g, 418.6 ± 40.4 ng/g, and 480.8 ± 50.4 ng/g dry feces, respectively; Fig. 2). However, fecal androgen concentration differed ($P < 0.05$) between dry and wet seasons (380.5 ± 28.0 ng/g versus 483.9 ± 32.3 ng/g dry faces; Fig. 3).

There was no significant relationship between body weight, testicular volume, semen volume, sperm concentration, motility, progressive forward motility and sperm morphology, temperature and photoperiod. In addition, fecal androgen concentrations were not significantly correlated with temperature or photoperiod.

Table 3
Mean (±S.E.M.) and range of fecal androgen concentrations (ng/g dry feces) for individual jaguars

Animal	n[a]	Mean (±S.E.M.)	Range
A	92	811.5 ± 51.6	19.0–1974.0
B	25	566.0 ± 74.5	18.5–1445.7
C	92	601.1 ± 41.2	22.2–1759.8
D	53	515.2 ± 67.6	14.6–1852.9
E	67	193.3 ± 33.2	13.2–1676.1
F	126	88.90 ± 9.90	10.5–584.10

[a] Total number of samples/animal.

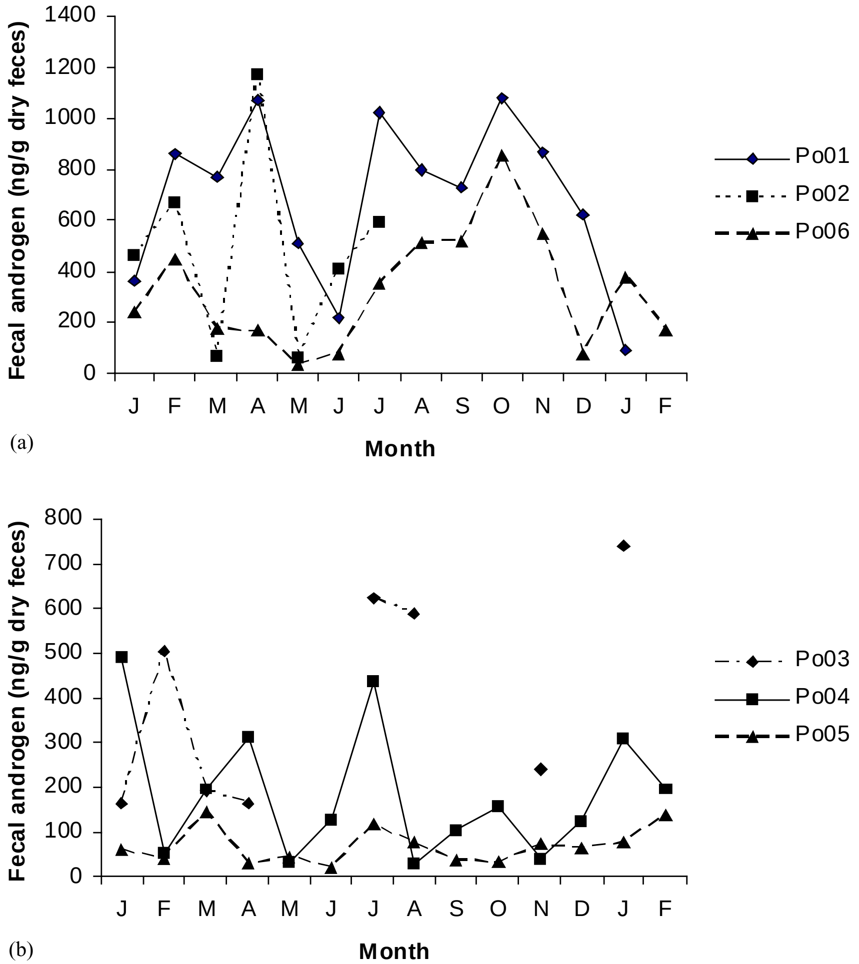

Fig. 1. Monthly means of fecal androgen excretion in jaguars in the São Paulo Zoo (a) and the Sorocaba Zoo (b).

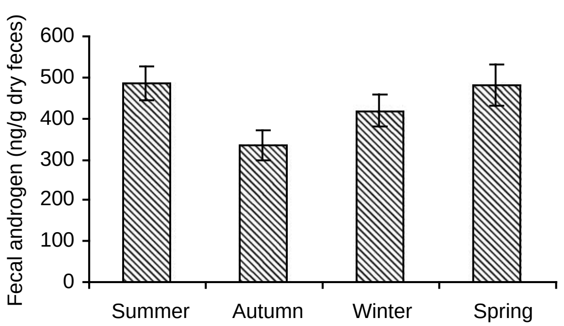

Fig. 2. Seasonal mean (±S.E.M.) of fecal androgen excretion in the jaguar.

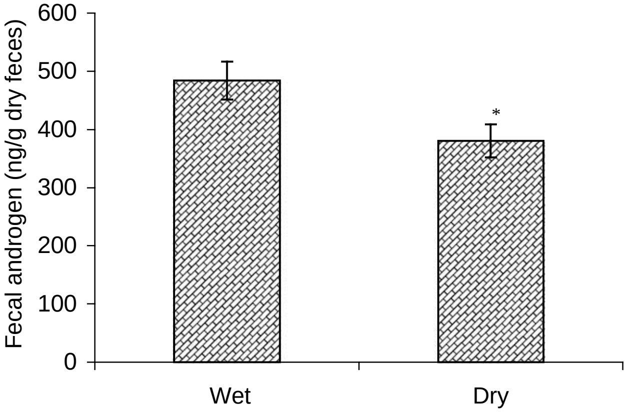

Fig. 3. Seasonal androgen excretion in captive jaguars (wet and dry seasons). Asterisk (*) denotes statistical difference ($P < 0.05$).

4. Discussion

Male jaguars did not have significant seasonal variations in the fecal androgen concentration, in agreement with previous findings of reproductive function in male neotropical small cats, including tigrina (*Leopardus tigrina*), ocelot (*Leopardus pardalis*) and margay (*Leopardus wiedii*) [16]. Similarly, in a previous report, serum testosterone concentration in jaguars did not vary throughout the year [12]. However, there was a significant difference in fecal androgen concentrations between the wet and dry seasons. In tropical species, the breeding season of many species coincides with periods of rainfall. However, the true stimulus is likely to be the availability of food and not rainfall itself [17,26]. While free-living jaguars have been reported to reproduce throughout the year in the tropics [18], there is an increase in the number of births between March and June in the southern Pantanal [19]. This period of increased frequency of jaguar births coincides with the receding of floodwaters when conditions are optimal for vegetative growth, resulting in an increase in the prey-base population. Perhaps an increase in parturition during the dry season is an evolutionary strategy to take advantage of increased food availability for growing litters and to avoid the energy-expensive period of lactation [20,21] during the wet season when food availability is low [19]. However, since the animals in the present study received the same diet throughout the year, there may be other factors responsible for seasonal differences.

In neotropical small cats there is a propensity for greater sperm production and androgen concentration during the summer months, coinciding with the longest photoperiod [16]. However, there was no significant relationship between sperm traits and environmental data in the present study. Similarly, we were unable to determine the factor(s) that affect the endocrine–testicular function; there was no correlation between fecal androgen concentration and photoperiod or temperature. Further research, preferably with a larger number of animals and conducted over at least a 2-year interval, is needed to provide more definitive information.

Semen quality was similar to previous studies [11,12]. There were no significant differences between the first and second collections, consistent with previous reports that semen quality in jaguars is fairly consistent throughout the year [11,12].

Differences between the two locations in the present study could have been due to management. Although environmental conditions were similar, two males at the Sorocaba Zoo (D and E) were housed together and one male was housed with a conspecific female (F). Like most cats, jaguars are solitary and territorial under natural conditions [22]. Housing these animals in groups and without appropriate hiding areas can increase stress [23] and therefore affect the reproductive performance, as described for small cats [24,25]. Persistent elevations in peripheral cortisol concentrations can indicate chronic stress [23]. Therefore, comparison of cortisol concentrations between groups would provide information on stress levels and potential reproductive problems arising from specific enclosures and environmental conditions. Although stress may have influenced the testicular activity of some males in this study, it is noteworthy that there was no significant difference between locations for any semen characteristic.

In summary, based on measurement of fecal androgens, jaguar males maintained androgen activity throughout the year, with peak production during the wet season. Future studies are necessary to improve the understanding of the seasonal influence on reproductive activity of free-living animals, sexual maturity in captive and free-living jaguars, the ability to evaluate the influence of housing conditions on the reproductive performance of captive jaguars, and the influence of human activity on free-living jaguar populations.

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