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Reproductive characteristics of captive male jaguars (*Panthera onca*)*

Características reprodutivas de onças-pintadas (*Panthera onca*) machos, mantidos em cativeiro

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SUMMARY

Ejaculate traits, testicular volume and plasma testosterone levels were determined once every two months for one year in 4 adult male jaguars (*Panthera onca*) housed at the São Paulo Zoo, SP, Brazil. Semen samples was collected by electroejaculation and analyzed for pH, total volume, motility (0-100%), status (0-5), total spermatozoa count and morphology. Blood samples was obtained by cephalic venipuncture immediately before the onset of electroejaculation and stored at –20°C until assay of testosterone by RIA. Using a calliper, the length and width of each testis was measured and the values were combined to determine the testicular volume. Semen analyses demonstrated a high percentage of structurally abnormal sperm (mean = 51%) and low rates of motility (50.6%) and status (2.2). No correlation was found between semen traits, plasma testosterone and testicular volume (Spearman's test). No season variation was detected throughout the year for semen traits, plasma testosterone and testicular volume ($p>0.05$, Friedman's test). The results of this study suggest that the captive jaguars in Brazil are not seasonal, and that semen collections can be performed throughout the year without a perturbation in overall ejaculate quality. However, underlying causes of high percentages of structurally abnormal sperm, found in captive jaguars, need to be investigated mainly to improve semen quality.

UNITERMS: Jaguars; Semen characters; Testosterone; Season.

INTRODUCTION

The jaguar (*Panthera onca*), the largest cat of the Americas, inhab its different regions from North Mexico to Argentina[19].In Brazil, the jaguar is considered an endangered species by the Brazilian Institute for Environmental and Natural Resources (IBAMA), and numbers in the wild have been reduced by a variety of factors with loss and fragmentation of habitats being as the most prevalent. In captivity, low reproductive success and inbreeding, due to small populations' size, has been a barrier for the genetic management of this species. Recently Morato; Gasparini[16] observed that 90% of the captive population of Brazilian Zoos is of unknown origin. Considering the importance of captive propagation to the preservation of endangered species, reproduction of wild caught and jaguars of known origin needs to become an integral component of conservation efforts for this species. Assisted reproductive technologies, such as artificial insemination and *in vitro* fertilization, can be viewed as one potential approach for safeguarding species, based on the highly successful applications to humans and domestic livestock[28]. However, to apply assisted reproductive strategies in the management of the jaguar, we need to first understand basic reproductive characteristics. Towards this aim, the objectives of this study were: 1) to examine the

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testicular function (steroidogenic and spermatogenic), and 2) to analyze the influence of season on reproductive activity in males.

MATERIAL AND METHOD

Animals

Four adult (7-10 years) male jaguars were maintained at the São Paulo Zoo, São Paulo, Brazil (23°34'09.5" S and 46°44'19.1" W). All males were not proven breeders. They were housed individually having access to indoor and outdoor enclosures and were exposed to the natural photoperiod throughout the year. A beef-based diet was provided 6 days a week.

Electroejaculation, collection of blood samples, semen evaluation and testicular biometry

Once every two months (July/1995 to July/1996) (n = 7 evaluations per male), males were anesthetized (10 mg/kg tiletamine-zolazepan; Zoletil®, Virbac, Brazil) by blow darting. Semen was collected by electroejaculation using a standardized technique[26]. In brief, a rectal probe (diameter, 2,4 cm; length 29 cm) and electrostimulator (AC, 60 Hz sine-wave; Eletrovet, São Paulo, SP, Brazil) were used to deliver a regimented sequence of electrical stimuli in an on-off pattern divided into 3 series. Series 1 and 2 consisted of 30 stimuli at 2, 3, 4 volts (series 1) and 3, 4, 5 (series 2). A total of 20 stimulations was given in series 3, with 10 stimuli each at 5 and 6 volts.

Semen from each collection was immediately evaluated for total volume and pH (reagent strips-Merck). Motility (0-100%) and rate of forward progression (status; 0-5) were based on observations of four separated microscopic fields at 100X[9,26]. An aliquot was diluted (1:3) in a 10% formaline-saline solution and used to determine the sperm concentration using a hemacytometer (Neubauer). Sperm morphology evaluations were performed after fixing an aliquot in a formaline-saline solution and examining 200 individual sperm cells using phase contrast microscopy (1,000X).

Blood samples (10 ml) were collected by cephalic venipuncture, immediately before the onset of electroejaculation. Samples were centrifuged at ambient temperature (300 g/10 minutes) 1 hour after collection, and the recovered sera stored at –20°C until hormone analyses by radioimmunoassay.

Clinical evaluations of the testicles were made by digital palpation and then classified as rigid, normal and flaccid. Using a calliper, the length and width of each testis was measured and the values were plotted in a formula ($V = LxW^2x0.524$, where L is the length and W is the width) to determine the testicular volume[11]. The volumes for the right and left testis were combined to obtain the total testicular volume of each male.

Radioimmunoassay

Plasma testosterone was measured as described by Lox *et al.*[12], using an antibody produced at the Unidade Edócrina of the Escola Paulista de Medicina of the Universidade Federal de São Paulo[21,25]. Tritiated testosterone (1, 2, 6, 7, 16, 17-^{3}H testosterone, approximately 1,000 c.p.m., New England Nuclear, Boston, MA, USA) was added to 0.5 ml of plasma sample for the recovery assay.

The tubes were incubated for 30 minutes at room temperature (25°C) and the testosterone extracted with 3 ml of twice-distilled ether (Merck, USA). After incubation, the tubes were centrifuged and the extracts separated by freezing.

The ether fraction was evaporated in a water-bath at 50°C, and the residue was suspended in 2 ml gelatin buffer.

Table 1

Individual ejaculate traits, testicular volumes and testosterone concentrations (mean ± SEM) of captive male jaguars, São Paulo Zoo, 1995 to 1996.

	Male 1	Male 2	Male 3	Male 4
Ejaculate volume (ml)	7.4±0.6	10.1±3.3	4.8±0.6	11.5±2.3
	(4.9-8.6)[a]	(2.3-4.4)	(2.3-5.8)	(5.8-20.2)
Total spermatozoa count (x10^6)	70.8±7.9	107.3±65.0	19.3±7.9	8.8±2.1
	(4.9-91.1)	(7.8-364.0)	(6.2-50.3)	(3.0-14.4)
Spermatozoa motility (%)	65.6±4.8	67.5±8.7	59.4±5.9	64.2±6.2
	(47.0-73.0)	(33.0-82.0)	(37.0-70.0)	(50.0-82.0)
Spermatozoa status	2.9±0.2	3.0±0.4	2.75±0.3	2.6±0.3
	(2.2-3.2)	(1.3-3.6)	(1.6-3.5)	(1.9-3.7)
Testicular volume (cm^3)	45.3±3.9	41.6±1.4	36.4±2.3	38.2±1.6
	(38.9-60.6)	(38.3-46.7)	(30.1-44.4)	(35.0-44.0)
Testosterone (ng/dl)	336.6±56.1	231.0±17.3	236.6±52.6	221.2±20.1
	(114.0-412.5)	(198.0-299.0)	(155.5-445.0)	(172.0-285.0)

[a] range.

Table 2
Seasonal evaluation of ejaculate traits, plasma testosterone and testicular volume (mean ± SEM) in jaguars, São Paulo Zoo, 1995 to 1996.

	Winter	Spring	Summer	Fall
Ejaculate volume (ml)	10.9±4.3	9.0±3.7	7.9±0.8	6.0±0.9
	(2.3-23.0)[a]	(4.9-20.2)	(5.6-9.7)	(4.4-8.6)
Total spermatozoa count (x10^6)	107.5±86.4	20.3±9.7	40.6±19.4	37.9±18.0
	(3.1-363.9)	(7.1-49.0)	(7.8-91.1)	(5.2-86.0)
Spermatozoa motility (%)	66.0±10.0	73.5±4.8	50.0±7.4	67.1±5.8
	(36.6-81.6)	(61.6-82.5)	(33.3-69.1)	(50.0-73.3)
Spermatozoa status	2.8±0.4	3.3±0.2	2.1±0.3	3.0±0.3
	(1.6-3.6)	(2.8-3.7)	(1.3-3.0)	(2.0-3.5)
Testicular volume (cm^3)	36.6±2.6	38.2±1.1	41.9±1.4	44.9±5.9
	(30.1-42.7)	(35.4-40.1)	(38.3-44.4)	(33.5-60.6)
Testosterone (ng/dl)	324±60.4	287.9±52.5	243.6±57.0	170.0±20.7
	(198.0-445.0)	(155.5-412.0)	(172.0-412.5)	(114.0-213.0)

[a] range. There is no significant variation of the analyzed parameters throughout the year (p>0.05).

Aliquots (0.1 ml) of this solution were transferred to scintillation tubes for the recovery assay and additional 0.1 ml aliquots were added to 0.1 ml of [1, 2, 6, 7, 16, 17-^{3}H]-testosterone (~7,000 c.p.m.) and 0.1 ml of antibody solution. The preparation was incubated at 4°C overnight and 0.2 ml of dextran-charcoal T-70 solution in gelatin buffer were added and mixed for 5 seconds.

The tubes were centrifuged at 800 g for 30 minutes at 4°C, and the supernatant was dispensed into a tube with 10 ml of scintillation liquid, and counted in a gamma counter. The assay sensitivity was 5.0 ng/dl and the net recovery was 90%. All samples were analyzed in duplicate with <10% intra- and inter-assay coefficients of variation.

Statistical analyses

The year was divided into four seasons that were represented for 1 or two months: winter (July), spring (September and November), summer (January) and fall (March and May). For each animal, mean (± SEM) values were calculated for seminal and hormonal characteristics obtained after each ejaculation procedure.

The data were then averaged across that season. The parameters for each animal during the seasons of the experiment were analyzed by the Friedman Rank Test using a statistical package (SSPS 6.0 for Windows, SSPS Inc., Chicago, IL). Spearman's rank correlation coefficient was calculated between seminal traits, testosterone concentrations and testicular volumes[7].

RESULTS

Based on a total of 28 collections, the mean ± SEM total ejaculate volume was 8.6 ± 1.3 ml (0.4-23.0), containing 3.9 ± 0.7 x 10^6 spermatozoa/ml (0-15.8) with a 50.6 ± 5.8% (32-82%) and 2.2 ± 0.3 (1.3-3.7) motility and status rating, respectively, and the pH 7. The ejaculate characteristics of each male are shown in Tab. 1. A high percentage of morphologically abnormal spermatozoa was present in the ejaculate of all males (mean =51%). The morphologic defects included macrocephaly (0.5%), microcephaly (1.5%), bicephaly (0.5%), narrow form heads (17.2%), round head (1.3%), abnormal acrosome (3.8%), knobbed acrosome (1.8%), abnormal midpiece (1.2%), no midpiece (0.3%), tightly coiled tail (5.2%), biflagellate (0.5%), bent midpiece with droplet (2.1%), bent midpiece without droplet (2.8%), bent tails with droplet (1.6%), bent tails without droplets (4.3%), proximal droplet (3.2%), distal droplet (2.5%), and bent neck (0.7%).

Ejaculate traits, testicular volume, and plasma testosterone concentrations were not significantly correlated (p>0.05).

Ejaculate traits, testicular volume and plasma testosterone concentrations were not statistically different for the various seasons (p>0.05) indicating no evidence of seasonal effects on these reproductive parameters (Tab. 2).

DISCUSSION

Semen collection, by electroejaculation, has been performed in many different cat species[9]. However, repeated collections have not been performed on jaguars. This study presents that repeated collections by electroejaculation in this species did not appear to affect semen quality, gonadal function or general health such as has been reported for tigers[3], snow leopards[11], Palla's cats[23] and others South American cats[15].

In general, semen analyses demonstrated a high percentage of structurally abnormal sperm. Although teratozoospermia is a common finding in felids[8] the percentages of abnormal sperm found in this study is higher than reported by others[9,14].

In general, three important factors have been directly identified to be related to semen quality, namely: genetic, nutrition and environmental conditions[1].

Considering the genetic factor, poor semen quality has been associated with inbreeding, has been reported for cheetahs[27] and Florida panthers[20]. Previous studies demonstrated that 90% of captive jaguars in Brazil are from unknown origin[16]; therefore, studies need to be conducted to elucidate the influence of reduced heterozygosity on the reproduction of captive jaguars in Brazil. Although the effect of nutrition on reproduction is complex, there is evidence suggesting that the beef-based diets fed to captive jaguars are inherently low in essential vitamins (e.g., A and E)[17]. Vitamin A and E deficiency has been associated with compromised spermatogenesis and testicular degeneration, respectively[10,13,22,24]. In light of these previous reports, studies need to be performed to evaluate the influence of essential nutrients on reproductive characteristics of captive male jaguars.

It is unlikely that the elevated number of morphologically abnormal spermatozoa in the jaguar was the result of sexual abstinence or degenerative process associated with elimination of aged spermatozoa once we performed two electroejaculations procedures in all jaguars before the beginning of the study. In this case, we observed similar number of defectives forms of spermatozoa.

Testicular volume has been used as an indicator of reproductive capacity and fertility in bulls[6]. Nevertheless, there was no apparent correlation between testicular volume and ejaculate traits in jaguars found in this study. Furthermore, there was no correlation between plasma testosterone concentrations and ejaculate traits, which was similar to that which was reported previously for Siberian tigers[3].

Seasonal variation was not detected in ejaculate traits, testicular volumes and plasma testosterone concentrations in captive male jaguars in this study. Based on the results of a behavioral study, Ewer[5] suggest that jaguars can reproduce throughout the year. Crawshaw *et al.*[4] and Morato *et al.* (unpublished data) observed male/female associations during different seasons in the Brazilian Pantanal. Jaguars inhabiting temperate areas in Mexico, however, have been reported to reproduce exclusively during the spring[18]. Relationship between latitude and the tendency to breed seasonally have been reported in rodents of the genus *Peromyscus*, two genera of medium size lagomorphs and deer of the genus *Odocoileus*[2].

It is possible that jaguars populations at different latitudes can be seasonal, however basic reproductive physiology needs to be investigated at different zoos locations and different natural habitats with special attention to factors that can influence the reproduction activity as food availability, rainfall and humidity, and ambient temperature.

The results of this study suggest that the captive jaguars in Brazil are not seasonal, and that semen collections can be performed throughout the year without a perturbation in overall ejaculate quality. However, high percentages of structurally abnormal sperm, found in captive jaguars, need to be investigated further to determine possible underlying causes. The outcome of this study is a first step towards the development of a genome resource bank and assisted reproduction program for the endangered jaguar.

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RESUMO

Quatro onças pintadas (*Panthera onca*), machos, adultos, mantidos no Zoológico de São Paulo (SP-Brasil), foram submetidos a avaliação seminal, quantificação hormonal e biometria testicular a cada dois meses pelo período de um ano. As amostras de sêmen foram obtidas por eletroejaculação e analisadas quanto ao pH, volume total, motilidade (0-100%), vigor (0-5), espermatozóides totais e morfologia. Amostras de sangue foram obtidas pela punção da veia cefálica imediatamente antes da eletroejaculação e o plasma foi estocado a –20°C até a realização do radioimunoensaio para quantificação de testosterona. Comprimento e largura dos testículos direito e esquerdo foram obtidos com auxílio de paquímetro e os valores obtidos foram combinados para a obtenção de volume testicular. As amostras de sêmen mostraram elevado índice de espermatozóides morfologicamente anormais (média = 51%), baixos índice de motilidade (50,6%) e vigor (2,2). Não foi encontrada correlação entre as características seminais, níveis plasmáticos de testosterona e volume testicular (Teste de Correlação de Spearman). Não foi detectado efeito da estação durante o ano para qualidade espermática, testosterona sérica e volume testicular (p>0,05, Teste de Friedman). Os resultados sugerem que as onças pintadas mantidas em cativeiro não são sazonais e que a colheita e avaliação espermática podem ser realizadas em qualquer período do ano sem que haja perda na qualidade espermática. No entanto, as causas de elevado índice de espermatozóides morfologicamente anormais, encontrados neste estudo, devem ser investigadas.

UNITERMOS: Jaguar; Características do sêmen; Testosterona; Sazonalidade.

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