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Morphology, morphometry, ultrastructure, and mitochondrial activity of jaguar (*Panthera onca*) sperm

Herlon Victor Rodrigues Silva[a,*], Thalles Gothardo Pereira Nunes[a], Leandro Rodrigues Ribeiro[b], Luana Azevedo de Freitas[a], Moacir Franco de Oliveira[c], Antônio Chaves de Assis Neto[d], Alexandre Rodrigues Silva[e], Lúcia Daniel Machado da Silva[a]

[a] Laboratory of Carnivores Reproduction, State University of Ceará, Fortaleza, CE, Brazil
[b] Haras Claro Farm - Caucaia-CE, Brazil
[c] Multiplication Center of Wild Animals, Federal Rural University of Semi-Arid, Mossoró, RN, Brazil
[d] Department of Surgery, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, SP, Brazil
[e] Laboratory of Animal Germplasm Conservation, Federal Rural University of Semi-Arid, Mossoró, RN, Brazil

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ABSTRACT

The jaguar is categorized as "Near Threatened". Conservation strategies, therefore, are needed which include use of reproductive biotechniques. For implementation of biotechnique use, the reproductive characteristics of the species must be understood, which is currently not the case. This study, therefore, aimed to describe the detailed morphology of jaguar sperm, and to evaluate the sperm mitochondrial activity. Five male adults were used. Slides stained with Rose Bengal were used for morphometric and morphological analyses. The length and the width of the sperm head were measured, as well as the length of the middle piece, the tail, and the total length. Scanning and transmission electron microscopy were used for ultrastructural analysis. Mitochondrial function was assessed using the marker 3,3′-diaminobenzidine (DAB). The results are expressed as means ± SEM. The most significant morphological abnormalities observed were head (9 ± 1.7%) and tail defects (12.5 ± 3.3%). The width and length of the head were 3.6 ± 0.03 µm and 4.9 ± 0.02 µm, respectively. The middle piece measured 9.7 ± 0.3 µm, the tail measured 54.5 ± 4.4 µm, and the total length of the sperm was 59.5 ± 0.1 µm. Electron-lucent regions and approximately 54 mitochondrial spirals in the middle piece were identified in the nucleus using electron microscopy. The greatest percentages of cells were classified as DAB I (46.6 ± 4.9%) and DAB II (38 ± 4.4%). The data provide detailed information on the sperm characteristics of jaguars and can support research on germplasm conservation for the species.

1. Introduction

The jaguar (*Panthera onca*) is monotypic within the genus *Panthera*, which is native to the American continent. The geographical distribution initially extended from the southern United States to northern Argentina, but at present its natural habitat is confined to small fragmented areas of the Central America, Mexico, and South America (Quigley et al., 2017). Habitat destruction, combined with predatory hunting, has caused populations to markedly decrease and the species is now considered "Near Threatened" according

* Corresponding author.
E-mail address: herlonvrs@hotmail.com (H.V.R. Silva).

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to the IUCN Red List (Quigley et al., 2017).

Consequently, conservation strategies which include use of the reproductive biotechniques are needed to preserve the species. To identify the most suitable biotechniques, however, the reproductive physiology of each species must be understood (Silva et al., 2004). Characterization of sperm is essential for the improvement of assisted reproduction techniques, contributing to the creation of biobanks that enable the preservation of the genetic material of the species (Silva et al., 2015).

One of the main methods for evaluating sperm is morphological analysis, in which the physiological structure of the cell can be determined, as well as possible anomalies that might affect fertilization (Chemes and Sedo, 2012). One such method is ultrastructural evaluation, which can also predict the disposition and integrity of the cell organelles, and can allow the observation of possible abnormalities that may go unnoticed with conventional light microscopy (Chemes and Rawe, 2003). In the Felidae, this technique has already been used to ascertain the potential abnormalities in the sperm of domestic cats and tigers (*Panthera tigris altaica*) (Schmehl and Graham, 1989); however, the ultrastructure of jaguar sperm has not been previously studied.

Morphometric analysis is another method used to evaluate morphologyand there can be use of this approach to determine sperm defects occurring mainly in head, including macro or microcephaly (Maree et al., 2010). Another important application for sperm morphometry in wild animals is to provide reference values for species that can be used in computer-assisted sperm analysis software (CASA), because most of these analyses do not include references for all the species (Soler et al., 2017).

Evaluation of mitochondrial function can predict the quality of sperm motility, given that the potential for movement of the cell is dependent on metabolic energy resulting from mitochondrial functions (Marchetti et al., 2004). The most common method used to accomplish this analysis is through use of fluorescent probes and a microscopes suitable for this type of analysis; however, for free-living wild animals or those in zoos a feasible method of sample preparation is needed at the site of collection for subsequent assessment. Accordingly, cytochemistry using 3,3′-diaminobenzidine (DAB) dye is an alternative technique to assess mitochondrial functions (Brito et al., 2018).

The present study, therefore, was conducted to describe for the first time the sperm structure of the jaguar, and provide information on the sperm morphology, morphometry, and mitochondrial functions.

2. Materials and methods

2.1. Animals

The study was approved by the Ethics Committee of the State University of Ceará (Nº 5098414/2016) and by the System of Authorization and Information of Biodiversity (Sistema de Autorização e Informação da Biodiversidade – SISBIO) (no. 54741–1). Five male jaguar weighing 70.8 ± 5.9 kg and ranging from 4 to 17 years of age were used. All the animals were housed in a zoo. There was one jaguar from each of the following that was used in the study: Ecological Park – EcoPoint, Fortaleza, CE, Brazil (3° 43′ S, 38° 30′ W); São Francisco Zoo, Canindé, CE, Brazil (4° 21′ S, 39° 18′ W); Teresina Zoobotanical Park, Teresina, PI, Brazil (5° 05′ S, 42° 48′ W); Arruda Câmara Zoobotanical Park, João Pessoa, PB, Brazil (7° 06′ S, 34° 51′ W); and Dois Irmãos Park, Recife, PE, Brazil (8° 03′ S, 34° 52′ W). All the zoos were located in the intertropical zone in the Brazilian northeast and, therefore, the animals were not subject to a possible reproductive seasonality due to differences in photoperiod at the various zoos. Each animal was subject to the usual management practices of each institution, with the regular provision of red meat or slaughtered chicken, as well as vitamin supplementation. Water was provided *ad libitum*. There is listing of further information regarding each individual animal in Table 1. All animals were captured in their natural habitat before being housed in the zoos that were previously described. Males 1, 2 and 5 were captured in locations of the Amazon Forest and Males 3 and 4 from the region of the Atlantic Forest. All animals were classified as being healthy and free of disease. None of the males had produced offspring because of matings before the time that the present study was conducted. All males had penile spicules, which characterize male hormonal stimulations of these tissues had occurred (Fig. 1 A–E).

2.2. Anesthesia

With each procedure, the animals were chemically restrained using blowgun darts containing dexmedetomidine (Dexdomitor ®, Zoetis, Campinas – SP, Brazil) at a dose of 0.04 mg/kg, IM, combined with ketamine hydrochloride (Ketalar®, Pfizer, São Paulo-SP, Brazil) at a dose of 5 mg/kg, IM. Fifteen minutes after administration of the combined drug the animals were in a decubitus position and, when necessary, a third of the initial dose was administered to maintain anesthesia. The total time to perform the anesthetic safety procedure was approximately 1 h. To reverse the anesthesia after semen collection, yohimbine was administered at a dose of

Table 1
All data about jaguars (*Panthera onca*) used.

Animals	City located	Age (Years)	Weight (Kg)	Housed with female	Origin
Male 1	Fortaleza/CE	17	66	Yes	Captive
Male 2	Canindé/CE	6	81	Yes	Captive
Male 3	Recife/PE	8	70	No	Captive
Male 4	João Pessoa/PB	9	69	Yes	Captive
Male 5	Teresina/PI	4	68	Yes	Captive

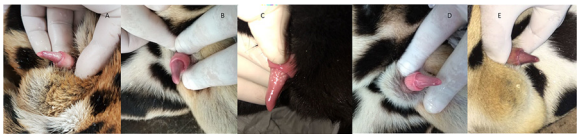

Fig. 1. Genitals of males, testicles and penis. (A) Male 1; (B) Male 2; (C) Male 3; (D) Male 4; (E) Male 5; Note penis in conical format, with approximate size of 5 cm and spicules present in penile base.

0.4 mg/kg, IM, and after a few minutes the animals were conscious (Lueders et al., 2012).

2.3. Semen collection

Semen was collected from the five animals between March and September 2017. Two collections were performed per animal (n = 10), with a minimum interval of 2 months between collections for each animal.

The preparation for semen collection began as soon as the animal was under the effects of anesthesia. The jaguar was placed in a lateral recumbency position (Fig. 2A) and the penile region was cleaned with saline solution (0.9%). Urine was completely expelled using a urethral probe, and three washes were performed by injecting and removing 10 mL of saline solution (0.9%), using the probe. The washing was performed slowly to avoid traumato the urethral canal, as well as reduce the possibility of contamination of the semen with urine (Fig. 2B; Curren et al., 2013).

Feces were then removed from the rectum of the animal and lubricating gel was used to facilitate the penetration of the electroejaculation probe which was inserted until reaching the prostate close to the rectal area (Fig. 2C), with the electrodes positioned towards the prostatic surface. Electroejaculation was performed using an electro-mechanical device (Autojac V2®, Neovet, Uberaba, MG, Brazil). The electrode probe had three 3-cm-long metal electrodes (positive, neutral, and negative). The procedure consisted of three sets of stimuli, with ten stimuli for each voltage, the first series starting with 5 V, 6 V, and 7 V; the second with 6 V, 7 V, and 8 V; and the third with 8 V and 9 V (Wildt et al., 1983). Each stimulus lasted for 2 s, with 5-minute intervals between each series. Samples were collected in 50-mL graduated tubes in which it was possible to completely insert the penis of the animal. The semen was evaluated immediately after collection (Fig. 2D).

2.4. Semen analysis

The semen was initially evaluated for the macroscopic parameters of volume and color, as well as the microscopic parameters of sperm motility (%) and vigor (scale 0–5; 0: all sperm without movement; 1: slowly lateral-lateral movements, without progression; 2: fast lateral-lateral movements, without progression; 3: rapid lateral-lateral movements, with occasional progression; 4: slow and continuous progression; 5: fast and continuous progression -Christiansen, 1984). The sperm concentration was determined from a 5 µL aliquot of semen diluted in a 1% formol saline solution, and subsequently evaluated using a light microscope at 400× magnification and a Neubauer chamber was used for cell counting. To evaluate sperm morphology, samples were fixed using 10 µL of the samples diluted in 90 µL Rose Bengal (distilled water 20 mL; sodium citrate 0.58 g; formaldehyde 0.8 mL; Rose Bengal 0.3 g; CAQ – Casa da Química, São Paulo-SP, Brazil) and observed using a light microscope at 1000x magnification (Cardoso et al., 2005). For each sample, 200 sperm were used to determine the proportion of normal to defective cells and to characterize the defects at different

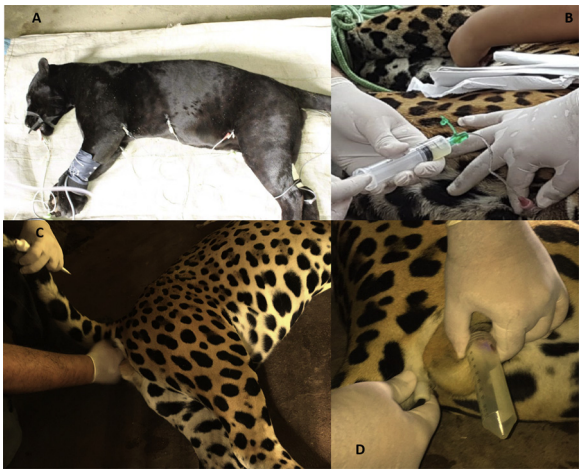

Fig. 2. (A) Lateral recumbency position, before semen collect; In male 3; (B) Removal of urine before semen collect; in Male 1; (C) Feces removal, before to insertion of electroejaculator probe; in Male 5; (D) Electrobejaculator probe, inserted into the rectus of animal, to start the electrical stimuli; Male 5.

locations (acrosome, head, middle piece, or tail) of the sperm cell (Wildt et al., 1986). The functionality of the sperm membrane was determined using a hypoosmotic test in which 10 μL of semen were diluted in 90 μL distilled water. The sample was subsequently stored at 37 °C for 40 min, followed by evaluation of the cell membrane of the cells that reacted positively (tail winding), or negatively (tail completely straight) (Silva et al., 2015; Lima et al., 2016).

2.5. Analysis of mitochondrial function

A 20 μL volume of semen was added to a 50 μL solution containing 15 mg/mL 3,3′-diaminobenzidine (DAB) diluted in phosphate buffered saline (PBS) and incubated in a water bath at 37 °C for 40 min, protected from light. Two 10 μL smears of each sample were prepared on microscope slides and dried at ambient temperature, fixed in 10% formaldehyde for 10 min, rinsed in distilled water, and then dried at ambient temperature (20–25 °C) (Hrudka, 1987). A total of 100 sperm were counted using a phase-contrast microscope (400 ×), and classified as class I DAB (100% of the middle piece was stained, indicating relatively greater mitochondrial function); class II DAB (> 50% of the middle piece was stained, indicating average mitochondrial function); class III DAB (< 50% of the middle piece was stained, indicating relatively lesser mitochondrial function); and class IV DAB (absence of staining in the middle piece, indicating the absence of mitochondrialfunction). The values obtained in each class were expressed as a percentage (Flores et al., 2016).

2.6. Morphometric analysis

The slides prepared previously for the morphological evaluation, stained with Rose Bengal, were used for morphometric analysis. Images of 200 normal sperm cells were recorded at random fields in each semen sample, and evaluated using a light microscope (1000x) connected to a computer. Cells were measured using image analysis software (ImageJ Software, Wayne Rasband–National Institute of Health, Maryland, United States).

The following sperm variables were measured: sperm head (width and length), middle piece (length), tail (length), and sperm (total length). The length of the sperm head was measured from the apex of the acrosome to the base of the head, and the width was measured along the transverse axis of its largest diameter. The length of the middle piece was measured from its insertion site at the base of the head to the region of the annulus (Jensen's ring). The length of the tail was measured from the beginning of the middle piece to the end of the caudal portion. The total length of the sperm was measured from the apex of the acrosome to the end of the caudal portion (Silva et al., 2015).

2.7. Analysis by scanning electron microscopy (SEM)

Scanning electron microscopy was performed using 5 μL aliquots of pooled semen samples from each animal. The samples were fixed in a 2.5% glutaraldehyde solution buffered in 0.1 M phosphate buffer (pH 7.4) and subsequently fixed in osmium tetroxide 1%. The samples were stored in a refrigerator at 4 °C until all the samples had been collected, after which the samples were processed for SEM evaluation at the Laboratory of Applied Animal Morphophysiology at the Federal Rural University of Semi-Árido. The samples were washed three times in 0.1 M phosphate buffer at pH 7.4 and twice with distilled water, followed by treatment with 1% tannic acid and dehydration with an alcohol series (50%, 70%, 90%, and 100%). After dehydration, drying was performed using a critical point apparatus with carbon dioxide as the atmosphere. The material was subsequently assembled in the sample holder (stub) and finely coated with gold through cathode pulverization, and then observed using a scanning electron microscope (LEO VP® 435 – Carl-Zeiss, Oberkochen, Germany; Silva et al., 2015).

2.8. Analysis by transmission electron microscopy (TEM) data

After each collection, a 5 μL sample of semen from each animal was fixed using a modified Karnovsky solution containing 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 (PB-Co® Sigma, St. Louis, United States) and kept in a refrigerator at 4 °C. The samples were submitted for processing at the Electron Microscopy Laboratory of the University of São Paulo (Faculty of Medicine – Campus of Ribeirão Preto, SP, Brazil). The fixed samples were washed with phosphate buffer and then post-fixed with 1% osmium tetroxide, dehydrated in propylene oxide, and embedded in Epon resin (Embed 812, Electron Microscopy Sciences, Hatfield, United States). Ultrathin sections (60–70 nm) were stained manually with uranyl acetate and contrasts were detected by using lead citrate. The samples were subsequently evaluated with a transmission electron microscope at the *Centro Avançado em Diagnóstico por Imagem (CADI)* of the School of Veterinary Medicine and Animal Science (FMVZ) of the University of São Paulo (USP), São Paulo, SP, Brazil. Different fields were selected randomly and evaluated using TEM (JEOL 1010, Japan), and images were recorded for later analysis and description (Silva et al., 2015).

2.9. Statistical analysis

The data were analyzed using the statistical software R-project© version 3.3.2 (The R Foundation, Vienna, Austria), being submitted to the Cramer-Von Mises normality test and the Box-Cox Homoscedasticity test. For comparison of means, the data were submitted to the Kruskal Wallis test followed by Dunn's test, with a confidence interval of 95%. The results are expressed as mean ± standard error.

Table 2
General seminal parameters for jaguars (*Panthera onca*). Values (Mean ± SEM), (n = 10; 2 ejaculates/male).

Male	Total motility (%)	Vigor (0-5)	Volume (mL)	Concentration (spz/mL) x 10^6	Functional membrane (%)
1	95 ± 0.0	5 ± 0.0	7.5 ± 2.5	120 ± 40.0	84 ± 2.0
2	92.5 ± 2.5	4.75 ± 0.3	5.5 ± 4.5	150 ± 50.0	70 ± 0.0
3	95 ± 0.0	4.75 ± 0.3	5.5 ± 1.5	75 ± 5.0	85 ± 2.0
4	95 ± 0.0	5 ± 0.0	6.3 ± 1.3	220 ± 120.0	84 ± 2.0
5	87.5 ± 7.5	4.5 ± 0.5	6.5 ± 0.5	145 ± 5.0	87.5 ± 2.5
Total	**93 ± 1.5**	**4.8 ± 0.1**	**6.25 ± 0.86**	**142 ± 25.68**	**82.5 ± 2.21**

* There was no statistical difference among the animals ($P > 0.05$).

3. Results

3.1. Semen evaluation

All semen samples were slightly cloudy, with translucent staining, and no urine contamination was observed in any of the ten assessed samples. The largest seminal volume was obtained during the period of use of the second cycle of electrical stimuli. The total volume of the ejaculate was 6.25 ± 2.7 mL, but there was wide variation in the volumes, varying from a minimum of 1 mL to a maximum of 10 mL, with both these samples at the range extremes being from Animal 2. Animal 5 had a greater variation in sperm motility and vigor, while the remaining animals had more homogeneous values. Animal 5, along with Animal 4, however, had the least variation in sperm concentration. All the samples were homogeneous with regard to membrane functionality, with an average of 82.5 ± 2.21%. The semen variable values for all individuals are presented in Table 2.

Normal sperm morphology was observed in 76 ± 3.5% of the samples. Animal 1 had the greatest percentage of morphologically normal cells among all the animals assessed (82.5 ± 1.5%), and Animal 5 had the least (66 ± 11%; Table 3). Even though there was considerable variation in values, there were no significant differences in sperm morphology among the individual animals. Fig. 3 depicts a normal sperm and some of the primary abnormalities that were detected.

3.2. Evaluation of mitochondrial activity

To evaluate mitochondrial activity, the four DAB categories were compared using the data from the five animals. The greatest percentages observed were for DAB I and II sperm meaning of these cells there was at least 50% of the intermediate pieces that were stained, with values of 46.6 ± 4.9% for DAB I and 38 ± 4.4% for DAB II, followed by DAB III with 9.5 ± 1.4%, and the least values for DAB IV with 5.9 ± 1%. There, however, were individual differences that were similar to the same general comparison pattern, with DAB I and II being in the greatest percentages compared to the other classifications. The other values for each animal are presented in Table 4.

Table 3
Morphological evaluation in jaguars (*Panthera onca*) sperm. Values (Mean ± SEM), (n = 10; 2 ejaculates/male).

Characteristics	Male 1	Male 2	Male 3	Male 4	Male 5	Total
Normal sperm	82.5 ± 1.5	77.5 ± 4.5	73.0 ± 10.0	81.0 ± 11.0	66.0 ± 11.0	**76.0 ± 3.5**
Head defects	5.5 ± 0.5	14.5 ± 5.5	6.5 ± 3.5	6.5 ± 4.5	12.0 ± 1.0	**9.0 ± 1.7**
Detached	0.0 ± 0.0	0.5 ± 0.5	4.5 ± 4.5	0.0 ± 0.0	2.0 ± 2.0	**1.4 ± 0.9**
Micro	1.5 ± 1.5	10.5 ± 8.5	0.5 ± 0.5	3.0 ± 1.0	3.0 ± 2.0	**3.7 ± 1.8**
Macro	1.0 ± 1.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5	**0.4 ± 0.2**
Degenerate	2.5 ± 2.5	3.0 ± 3.0	0.0 ± 0.0	3.5 ± 3.0	6.5 ± 1.5	**3.1 ± 1.1**
Piriform	0.5 ± 0.5	0.0 ± 0.0	1.5 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	**0.4 ± 0.3**
Middle piece (MP) defects	2.5 ± 2.5	2.0 ± 2.0	0.5 ± 0.5	1.0 ± 0.0	1.0 ± 0.0	**1.4 ± 0.5**
Abaxial	1.5 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	**0.3 ± 0.3**
Incomplete	0.0 ± 0.0	1.5 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	**0.3 ± 0.3**
Thick	0.5 ± 0.5	0.0 ± 0.0	0.5 ± 0.5	0.5 ± 0.5	0.0 ± 0.0	**0.3 ± 0.2**
Reverse	0.5 ± 0.5	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	**0.2 ± 0.1**
Bent	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5	1.0 ± 0.0	**0.3 ± 0.2**
Tail defects	8.0 ± 0.0	3.5 ± 1.5	20.0 ± 7.0	10.0 ± 8.0	21.0 ± 12.0	**12.5 ± 3.3**
Very coiled	3.0 ± 3.0	0.5 ± 0.5	7.0 ± 2.0	2.0 ± 1.0	0.5 ± 0.5	**2.6 ± 1.0**
Coiled	5.0 ± 3.0	3.0 ± 2.0	13.0 ± 9.0	8.0 ± 7.0	20.5 ± 12.5	**9.9 ± 3.3**
Acrossomal defects	1.5 ± 1.5	2.0 ± 1.0	0.0 ± 0.0	1.5 ± 1.5	0.0 ± 0.0	**1.0 ± 0.5**
Broken	1.5 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	**0.3 ± 0.3**
Withdrawn	0.0 ± 0.0	1.5 ± 1.5	0.0 ± 0.0	1.5 ± 1.5	0.0 ± 0.0	**0.6 ± 0.4**
Fragmented	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	**0.1 ± 0.1**
Teratogenics	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	**0.1 ± 0.1**
Total defects	**17.5 ± 1.5**	**22.5 ± 4.5**	**27.0 ± 10.0**	**19.0 ± 11.0**	**34.0 ± 11.0**	**24.0 ± 3.5**

* There was no statistical difference among the animals ($P > 0.05$).

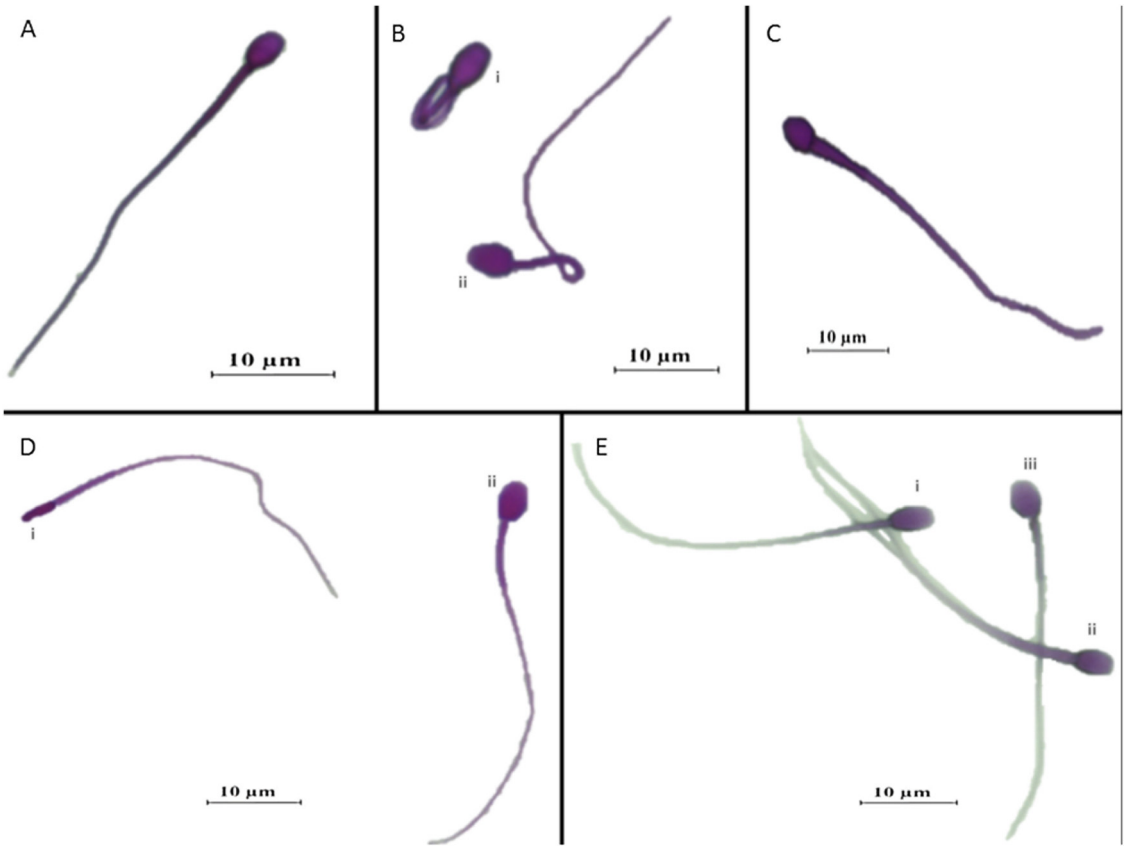

Fig. 3. Normal sperm morphology and sperm defects in jaguar (*Panthera onca*) - 1000x magnification. (A) Normal sperm (B) i - Very coiled tail; ii - Coiled tail (C) Thick middle piece (D) i - Micro head; ii - Normal sperm (E) i - Normal sperm; ii - Two tails; iii - Abaxial middle piece.

Table 4
Evaluation of mitochondrial activities in jaguar (*Panthera onca*) sperm through the use of 3,3′-diaminobenzidine (DAB). Values (Mean ± SEM), (n = 10; 2 ejaculates/male).

Male	DAB 1 (%)	DAB 2 (%)		DAB 3 (%)	DAB 4 (%)
1	51 ± 1.0^{a}	39 ± 1.0^{b}	6.5 ± 1.5^{c}		3.5 ± 1.5^{c}
2	58 ± 12.0^{a}	29 ± 5.0^{b}	7 ± 3.0^{b}		6 ± 4.0^{b}
3	36 ± 4.0^{a}	51 ± 5.0^{b}	9 ± 1.0^{c}		4 ± 2.0^{c}
4	44 ± 22.0^{a}	40 ± 22.0^{a}	9 ± 1.0^{a}		7 ± 1.0^{a}
5	44 ± 12.0 ^{a}	31 ± 7.0^{a}	16 ± 4.0^{a}		9 ± 1.0^{a}
Total	**46.6 ± 4.9^{A}**	**38 ± 4.4^{A}**	**9.5 ± 1.4^{B}**		**5.9 ± 1.0^{C}**

Lowercase letters comparing classes in each animal. Different superscript lowercase letters in the same row means that there was a statistical difference among DAB's classes ($P < 0.05$).
Uppercase letters comparing classes in total. Different superscript uppercase letters in the same row means that there was a statistical difference among DAB's classes ($P < 0.05$).

3.3. Sperm morphometric evaluation

The sperm of the jaguar had a head with a slightly oval shape, 3.6 ± 0.03 µm wide and 4.9 ± 0.02 µm long. The length of the intermediate piece was 9.7 ± 0.3 µm, while the total length of the tail was of 54.5 ± 0.1 µm. The total length of the sperm cell was 59.5 ± 0.1 µm, with homogeneity in individual values. The remaining results are shown in Table 5.

3.4. Ultrastructural evaluation of sperm by scanning and transmission electron microscopy

The images obtained using scanning electron microscopy were used to characterize the sperm of Jaguar. These sperm had a slightly oval head (Fig. 4A), nearly round, but with an easily distinguishable equatorial region dividing the acrosome hood (Fig. 4B). In the images obtained using transmission electron microscopy, there was characterization of a very electron-dense nucleus that was

Table 5
Values (Mean ± SEM) for sperm morphometry in in jaguars (*Panthera onca*) sperm, (n = 1000; 200 sperm cells/male).

Male	Head width (μm)	Head length (μm)	MP length (μm)	Tail length (μm)	Total length (μm)
1	3.1 ± 0.02[a]	4.6 ± 0.03[a]	9.2 ± 0.05 [a]	55.4 ± 0.3 [a]	60.1 ± 0.3 [a]
2	3.5 ± 0.03[b]	5.2 ± 0.03[b]	9.7 ± 0.07 [b]	54.3 ± 0.3 [a]	59.5 ± 0.3 [a]
3	4.1 ± 0.07[b]	4.9 ± 0.03[c]	9.3 ± 0.06 [a]	51.0 ± 0.2 [b]	56.0 ± 0.3 [b]
4	4.2 ± 0.08[b]	5.1 ± 0.04b[c]	10 ± 0.08[c]	54.9 ± 0.3 [a]	60.0 ± 0.3 [a]
5	3.1 ± 0.02[a]	4.7 ± 0.03 [a]	10.2 ± 0.06[d]	57.1 ± 0.3[c]	61.7 ± 0.3[c]
Total	**3.6 ± 0.03**	**4.9 ± 0.02**	**9.7 ± 0.3**	**54.5 ± 0.1**	**59.5 ± 0.1**

Different superscript lowercase letters in the same column means that there was a statistical difference ($P < 0.05$).

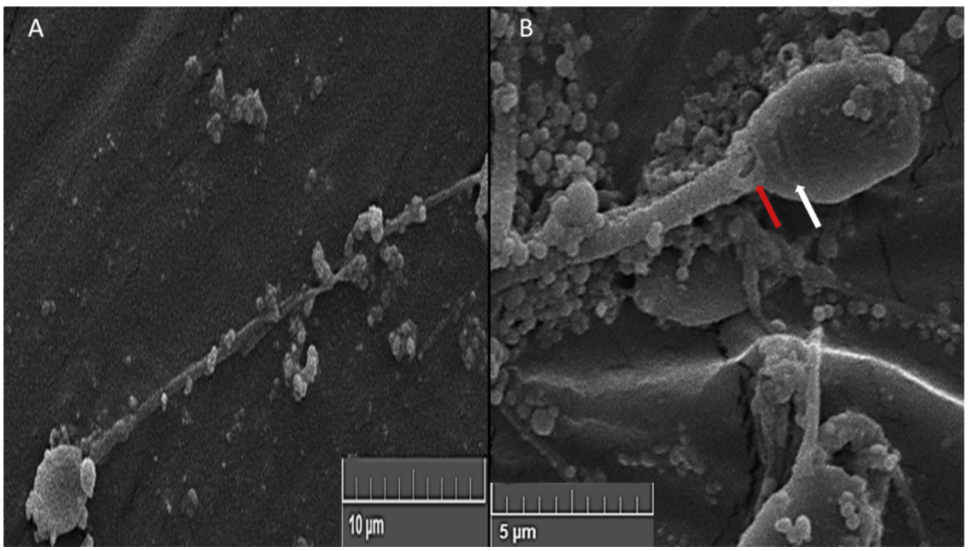

Fig. 4. Electron microphotograph of sperm morphology in jaguar (*Panthera onca*); (A) Sperm in a total visualization by microscopy scanning; (B) Head of the sperm - equatorial segment (white arrow); Neck area (red arrow) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

almost 100% homogeneous, as well as diffuse small electronic spots, in a longitudinal cut in the head of the sperm (Fig. 5A). In this region, besides a separated nucleus, the separation of the nuclear, and internal and external acrosomal membranes could be discerned, as well as the plasmalemma. In the basal end of the head, there was observation of a deployment pit of the middle piece and, in this region, the capitulum was detected below the proximal centriole. The first spiral of the mitochondria in the neck region could also be visualized. As shown in Fig. 5B, there was observation of a cross-section of the tail, containing the axoneme with a set of nine dense external fibers, surrounded by a mitochondrial ring. In Fig. 5C, there is observation of the section of the sperm from the head to the middle piece. In this section, there are approximately 54 mitochondrial spirals around the dense outer fiber along the length of the piece and extending to the region of the annulus, forming the mitochondrial sheath.

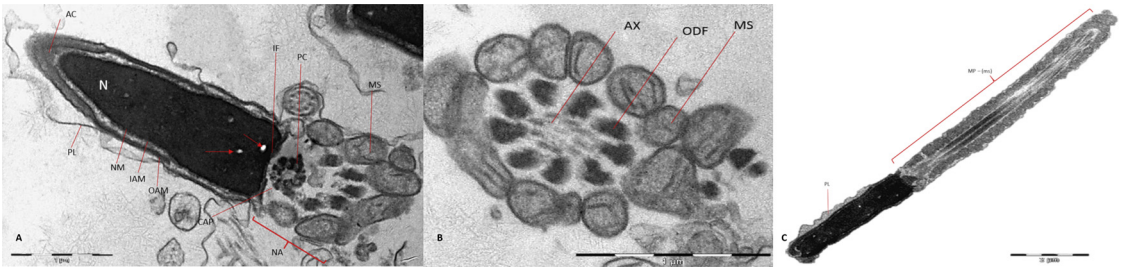

Fig. 5. Electron microphotograph of sperm morphology in jaguar (*Panthera onca*); (A) AC: acrosome; OAM: outer acrosome membrane; IAM: inner acrosome membrane; PL: plasmalemma; NM: nuclear membrane; N: nucleus; electron lucent nuclear dots (red arrows); NA: neck area; IF: implantation fossa; CAP: *capitulum*; PC: proximal centriole; ODF: outer dense fibers; MS: mitochondrial spiral; (B) AX: axoneme; MS: mitochondrial sheath; ODF: outer dense fibers; (C) Head and middle piece PL: plasmalemma; MP: middle piece (ms: mitochondrial spiral) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

4. Discussion

The semen collections in the present study were successful, with samples being obtained using a combination of dexmedetomidine and ketamine. In relation to the variables assessed, the percentage of the average total motility was high (93 ± 1.5%), which was also previously reported in results from other studies with jaguars (Paz et al., 2007; Araujo et al., 2017; Jimenez Gonzalez et al., 2017). Furthermore, the sperm vigor exceeded 3 on a scale of 0–5 (4.8 ± 0.1) indicating the samples collected were of excellent quality.

The volume of ejaculate was similar to that reported from results in previous studies using electroejaculation in jaguars (Paz et al., 2007), and the average sperm concentration was very high compared to values reported in previous studies where there was use of electroejaculation. This can be explained by the use of dexmedetomidine which increases the potential for erection, and consequently for ejaculation, due to its pharmacokinetic actions of this drug.; With use of the electroejaculation approach conducted in the present study, sperm count in the ejaculate, and cells with a functional membrane were both greater than 70% (Swanson et al., 2017).

A high percentage of sperm cells in the Felidae have morphological defects (Wildt et al., 1986; Axnér and Linde Forsberg, 2007), although this value may vary widely in some species, and can also be affected by the seasonal reproduction period (Swanson et al., 1996). There, however, was observation of a relatively greater percentage of normal cells (76.0 ± 3.5%) in the present study, in which there were the greatest percentages of defects in the tail (12.5 ± 3.3%) and head (9 ± 1.7%) of sperm cells. The percentages of normal cells vary substantially in studies with jaguars; Paz et al. (2007) observed only 27.6%, while Araujo et al. (2017) observed 60.7% and 51% in captive and free-living animals, respectively. Araujo et al. (2017) also reported that the greatest percentage of defects was related to malformations of the sperm tail.

Morphometry is a morphological method to analyze sperm that can be used to determine changes in the size of a cellular structure, and assist in determination of a standard for sperm size of a species. In jaguar, the morphology of sperm cells was not big compared with that of other carnivores such as coatis (*Nasua nasua*; Silva et al., 2015), American black bear (*Ursus americanus*; Brito et al., 2010), and artic fox (*Alopex lagopus*; Soler et al., 2017). The sperm size of the jaguar is similar to that previously reported for the puma (*Puma concolor*; Cucho et al., 2016) with the length and width of the head being nearly the same, however, for the jaguar being slightly longer than it is wide. The size and shape of the sperm can greatly affect the function of the cell, including the capacity to undergo the acrosome reaction and bind to the zona pellucida; therefore, these characteristics may also affect the capacity to predict male fertility potential (Maree et al., 2010).

The main objective of assessing mitochondrial function is to ascertain the percentage of cells with the greatest motility potential (Hrudka, 1987). In this regard, the ejaculate *in natura* of jaguars indicated there was a high percentage of cells with mitochondrial activity, with most of the cells classified as DAB I and II, indicating there was mitochondrial function in greater than 50% of the intermediate piece. This finding may be associated with the high values obtained for the general variables evaluated in the seminal sample. This indicates there may be a positive correlation between the high mitochondrial activity, and the high percentage of motile cells as well as those with a high progressive motility index.

In the SEM analysis, the sperm of the jaguar had a slightly oval shape, tending toward a more rounded morphology. There have been similar results reported by Schmehl and Graham (1989) for tiger sperm (*Panthera tigris altaica*) which also had a rounded morphology, unlike the sperm of the domestic cat that has a more elongated head. This finding suggests that this sperm head shape is the most common in species belonging to the subfamily Pantherinae, because Wildt et al. (1986) described a more elongated sperm head in cheetahs (*Acinonyx jubatus*) which, together with the cat, belongs to the subfamily Felinae. The differences in head morphology suggest a way of preventing the penetration of the oocyte by sperm that are compromised from a morphological perspective (Schmehl and Graham, 1989).

The characteristics of the jaguar sperm cell that can be described from using TEM initially includes the nucleus, which had an almost completely uniform size, from the region closest to the neck up to the most apical zone. This characteristic is similar to that of other Felidae such as cats and tigers (Schmehl and Graham, 1989), but not that of other carnivores such as the coati (*Nasua nasua*; Silva et al., 2015), artic fox (*Vulpes lagopus*; Hofmo and Berg, 1989), mustelids (Van der Horst et al., 1991), dog (Silva et al., 2009), and American black bear (*Ursus americanus*; Brito et al., 2010). In these species the sperm has a thin end near the acrosome with enlargement along the structure resulting a triangular shape. This similarity in sperm structure among these species may be related to the phylogenetic proximity of these species in which there is greater morphological similarities among sperm cells of the different species due to greater phylogenetic proximity. Several electron-lucent regions were observed in the cell nucleus, similar to what has been reported in other carnivores such as the Americans **black** bear (Brito et al., 2010) and coatis (Silva et al., 2015). The presence of these regions is related to changes that may occur during spermatogenesis with development of areas of condensed chromatin that, together with chromatin stabilization, can serve as markers of sperm maturity (Lazaros et al., 2011). Mitochondria were observed to be located in the sperm middle piece with these organelles having a spiral formation around a longitudinal axis, where these organelles function to provide energy for the movement of the flagella, providing motility to the cell (Amaral et al., 2013). The orientation of the mitochondria in the jaguar is very similar to that already described for the tiger and cheetah (Wildt et al., 1986; Schmehl and Graham, 1989), where a circular orientation of the mitochondria from the neck area was described. The number of mitochondrial spirals in the jaguar sperm (54 spirals) is very similar to that previously described for coatis (55 spiral; Silva et al., 2015) and black bears (59 spirals; Brito et al., 2010).

In general, the jaguar has values for sperm variables that are indicative that sperm should have a very acceptable fertilizing capacity compared to other wild carnivores, especially other Felidae. Although the jaguar sperm is relatively small, it has almost the same amount of mitochondrial spirals as other carnivores with larger sperm. Together with a high potential for sperm mitochondrial functionality, this suggests that the sperm architecture may be associated with sperm that have a relatively longer motility period in

this species because larger sperm require more energy, and consequently mitochondria, for the motility functions (Levitan, 2000).

There were few significant differences in values for variables evaluated in individual animals, indicating a homogeneity of sperm in the samples from the different animals. The values for variables evaluated also provide a reliable estimate for the jaguar indicating the expected values that should be expected when collecting semen from this species. Furthermore, the typical ultrastructural sperm cell morphological characteristics ascertained in the present study for the jaguar provide important background information for future studies with this species. The morphometry results from the present study can serve as a basis for computer-aided semen assessment in the *P. onca* species, as well as a model for other species of the Pantherinae subfamily because of the similarities in cellular structure with phylogenetic proximity. Nevertheless, in the present study, it was not possible to obtain semen with use of pharmacological collection procedures from all the animals; therefore, electroejaculation was the method selected for semen collection for standardization purposes. The jaguar usually has excellent semen quality *in natura*, and has sperm cells with relatively greater mitochondrial function than some other species. The values for other variables assessed in the present study provide information about the ideal characteristics and possible sperm pathologies in this species. This information can be used to develop cryopreservation protocols, serving as a database, and consequently contributing to the conservation of the species.

Conflict of interest statement

The authors have stated that there are no competing interests. None of the authors has financial or personal relationships that may influence or distort the content of the article.

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