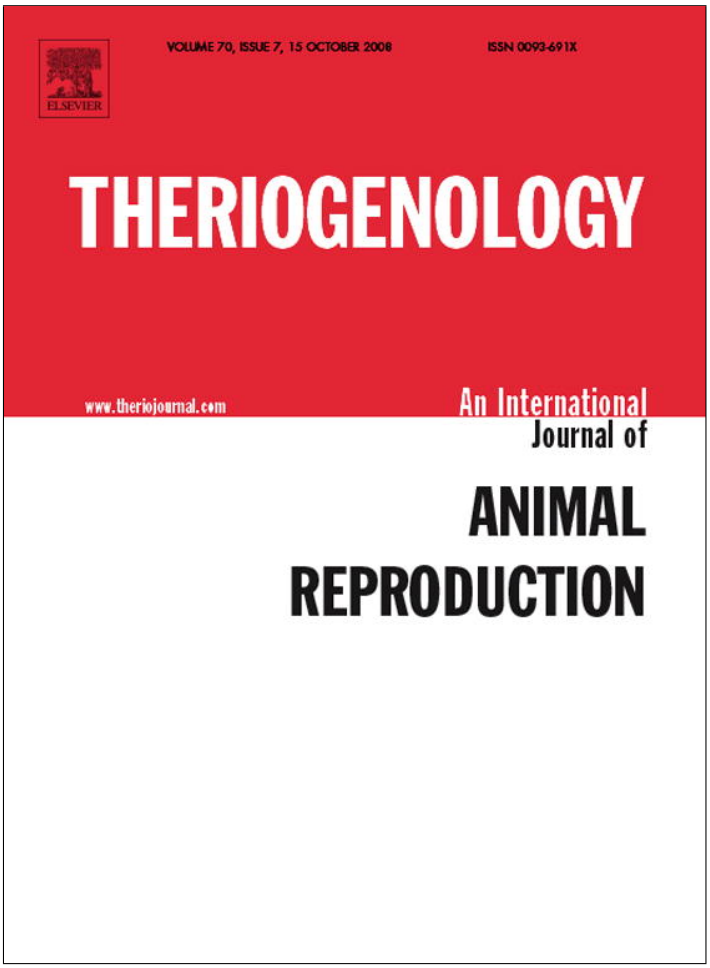

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Duration of spermatogenesis and daily sperm production in the jaguar (*Panthera onca*)

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Abstract

The jaguar, like most wild felids, is an endangered species. Since there are few data regarding reproductive biology for this species, our main goal was to investigate basic aspects of the testis and spermatogenesis. Four adult male jaguars were utilized; to determine the duration of spermatogenesis, two animals received an intratesticular injection of H^3-thymidine. Mean (±SEM) testis weight and the gonadosomatic index were 17.7 ± 2.2 g and 0.05 ± 0.01%, respectively, whereas the seminiferous tubules and the Leydig cells volume density were 74.7 ± 3.8 and 16.7 ± 1.6%. Eight stages of spermatogenesis were characterized, according to the tubular morphology system and acrosome development. Each spermatogenic cycle and the entire spermatogenic process (based on 4.5 cycles) lasted approximately 12.8 ± 0.01 and 57.7 ± 0.07 d. The number of Sertoli and Leydig cells per gram of testis was $29 \pm 4 \times 10^6$ and $107 \pm 12 \times 10^6$. Based on the number of round spermatids per pachytene spermatocyte (2.8 ± 0.3:1; meiotic index); significant cell loss (30%) occurred during the two meiotic divisions. There were approximately eight spermatids for each Sertoli cell (Sertoli cell efficiency), whereas the daily sperm production per gram of testis was $16.9 \pm 1.2 \times 10^6$. We expect that in the near future, the knowledge obtained in the present investigation will facilitate, utilizing germ cell transplantation, preservation of the germinal epithelium and the ability to generate sperm from jaguars in testes of domestic cats.

Keywords: Testis; Morphometry; Spermatogenic efficiency; Spermatogenic cycle length; Jaguar

1. Introduction

Modern felid species descended from relatively recent ($<11 \times 10^6$ years ago) divergence and speciation events that produced successful predatory carnivores worldwide [1]. Similar to most wild felids, the jaguar (*Panthera onca*), the largest felid in the American Continent, is endangered (http://www.iucnredlist.org/), currently threatened by habitat loss, fragmentation, and human persecution [2]. To worsen this situation, the knowledge of male reproductive function in the jaguar is very limited [3,4].

Spermatogenesis is a cyclic, complex and highly organized process in which diploid spermatogonia differentiate into mature haploid spermatozoa. This process is composed of cellular associations called stages, which may be classified according to the

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changes in the shape of the spermatid nucleus, the occurrence of meiotic divisions, and the arrangement of spermatids within the germinal epithelium [5–7]. Also, these stages can be identified based on the development of the acrosomic system and the morphology of developing spermatids [8–10]. The total duration of spermatogenesis, which requires approximately 4.5 cycles, lasts from 30 to 75 d in mammals [7,10], has been generally considered constant for a species [11], and is under the control of the germ cell genotype [12].

Germ cell transplantation is a fascinating and powerful technique that has been primarily used in the past decade for investigating spermatogenesis and stem cell biology in mammals [13–17]. This technique also offers great potential for studies involving biotechnology, transgenics, and the preservation of the genetic stock of valuable animals or endangered species [13–17]. In that regard, germ cell transplantation studies are being developed in our laboratory in the domestic cat; our objective is to use this species as a recipient model to preserve the genetic stock of wild felids, including the jaguar. For these investigations, knowledge of germ cell morphology and the duration of spermatogenesis are important [7,12].

There are few reports in the literature concerning the reproductive biology in jaguars [3,4]. In that regard, the main objectives of the present study were a detailed and comprehensive histological and morphometrical investigation of the testis and determination of the length of spermatogenic cycle and daily sperm production in the sexually mature jaguar.

2. Materials and methods

2.1. Animals

Four adult animals weighing 77 ± 3 kg were utilized. These animals were from zoos located in the cities of Rio de Janeiro and Porto Alegre (Brazil). As sperm production in jaguars and androgen metabolite concentrations in the captive male jaguar apparently were not affected by season [4], testis samples were not collected at any specific period of the year. Testes were separated from the epididymis and weighed, and manually cut longitudinally (with a razor blade) into small slabs, which were fixed by immersion in 4–5% buffered glutaraldehyde for 12–24 h. Tissue samples (2–3 mm thick) were routinely processed and embedded in plastic (glycol methacrilate) for histological, morphometric, and autoradiographic evaluation. Before surgery, all jaguars were treated i.m. with 10 mg/kg of a combination of zolazepam and tiletamin (Zoletil50; Virbac do Brasil, Ind. e com. LTDA, São Paulo, SP, Brazil). All surgical procedures were performed by a veterinarian and followed approved guidelines for the ethical treatment of animals.

2.2. Thymidine injections and tissue preparation

Before orchiectomy, intratesticular injections (75 μCi/testis) of tritiated thymidine (thymidine [methyl-3H], specific activity 82.0 Ci/mmol, Amersham, Life Science, England) were done close to the cauda epididymis cauda, in order to estimate the duration of spermatogenesis. Two time intervals were considered (1 h and 18 d) after thymidine injections. Tissue samples, 2–3 mm thick, were collected near the site of thymidine injections and routinely fixed and embedded, as described above.

To perform autoradiographic analysis, unstained testis sections (4 μm) were dipped in an autoradiographic emulsion (Kodak NTB-2, Eastman Kodak Company, Rochester, NY, USA) at 43–45 °C. After drying for approximately 1 h at 25 °C, the testis sections were placed in sealed black boxes and stored in a refrigerator (4 °C) for approximately 4 weeks. Subsequently, testis sections were developed in Kodak D-19 solution at 15 °C [18] and stained with toluidine blue. Analyses of these sections were performed by light microscopy to detect the most advanced germ cell type labeled at the two different time periods post thymidine injections. Cells were considered labeled when at least four or five grains were present over the nucleus in a low to moderate background.

2.3. Testis morphometry

The volume densities of the testicular tissue components were determined by light microscopy using a 441-intersection grid placed in the ocular of the light microscope. Fifteen fields chosen randomly (6615 points) were scored for each animal at 400× magnification. The tubular diameter and the seminiferous tubule epithelium height were measured at 100× magnification, using an ocular micrometer calibrated with a stage micrometer. Thirty tubular profiles, that were round or nearly round, were chosen randomly, and measured for each animal. The epithelium height was obtained in the same tubules utilized to determine tubular diameter. The total length of seminiferous tubule (meters) was obtained by dividing seminiferous tubule volume by the squared radius of the tubule multiplied by π [19].

2.4. Stages of the seminiferous epithelium cycle

2.4.1. Morphology system

Stages of the cycle in jaguars were characterized based on the shape and location of spermatid nuclei, presence of meiotic divisions, and overall seminiferous epithelium composition [5,6]. This method provided eight stages of the seminiferous epithelium cycle; their limits were quite similar to those stated by Amann [6]. The relative stage frequencies were determined from the analysis of 150 seminiferous tubule cross-sections per animal, at 400× magnification.

2.4.2. Acrosomic system

Stages of the seminiferous epithelium cycle were also characterized based on the development of the acrosomic system and morphology of the developing spermatid nucleus. In order to make a comparison between felids, this analysis was also performed in domestic cats (*Felis catus*), utilizing testicular tissue already available in our laboratory from other studies [20]. Our intention with this study was to compare potential morphological markers related to the development of the acrosome for the jaguar and the domestic cat. As noted in the results section, this method provided eight stages of the seminiferous epithelium cycle for both jaguars ($n = 4$) and domestic cats ($n = 25$). The relative stage frequencies were determined evaluating 150 seminiferous tubule cross-sections per each animal, at 400× magnification. When applied, for both species, the measurement of the angle of the acrosome on the nuclear surface was obtained from 150 germ cells (per animal and per stage of the cycle), at 1000× magnification.

2.5. Length of the seminiferous epithelium cycle

Both testes were analyzed for each animal. The histological sections utilized were those which were of better quality and had more tubular cross-sections. The duration of the spermatogenic cycle was estimated based on stage frequencies, characterized according to the tubular morphology system, and the most advanced germ cell type labeled at different times after thymidine injections. The total duration of spermatogenesis took into account that approximately 4.5 cycles are necessary for this process to be completed, from type A spermatogonia to spermiation [21].

This system was used to establish the duration of the cycle and spermatogenesis, daily sperm production, and efficiency of sperm production in this species, based upon histological and testicular measurements, in conjunction with radioautography.

2.6. Cell counts and cell numbers

This approach was performed based on the seminiferous tubules characterized according to the tubular morphology system. All germ cell nuclei and Sertoli cell nucleoli present at Stage 1 of the cycle were counted in ten round (or nearly round) seminiferous tubule cross-sections, chosen at random, for each animal. These counts were corrected for section thickness and nucleus or nucleolus diameter [22]. For this purpose, 10 nuclei or nucleoli diameters were measured (per animal) for each cell type analyzed. Cell ratios were obtained from the corrected counts obtained at Stage 1. The total number of Sertoli cells was determined from the corrected counts of Sertoli cell nucleoli per seminiferous tubule cross-sections and the total length of seminiferous tubules [23]. Daily sperm production (DSP) per testis and per gram of testis (spermatogenic efficiency) were obtained according to the following formula [24]: DSP = total number of Sertoli cells per testis × the ratio of round spermatids to Sertoli cells at Stage 1 × Stage 1 relative frequency (%)/Stage 1 duration (d).

Individual volume of the Leydig cell was obtained from nucleus volume and the proportion between nucleus and cytoplasm. Because the Leydig cell nucleus in jaguars was spherical, nucleus volume was calculated from mean nuclear diameter. For this purpose, for each animal, 30 nuclei with an evident nucleolus were measured. Leydig cell nuclear volume was expressed in μm^3 and obtained by the formula $4/3(R^3$, were R = nuclear diameter/2. To calculate the proportion between nucleus and cytoplasm, a 441-point square lattice was placed over the sectioned material at 400× magnification, and 1000 points over Leydig cells were counted for each animal. The total number of Leydig cells per testis was estimated from the Leydig cell individual volume and the volume occupied by Leydig cells in the testis parenchyma.

3. Results

3.1. Biometric data and testis volume density

The mean (±SEM) testis weight for the adult jaguar was 17.7 ± 2.2 g, providing a gonadosomatic index (testes mass divided by body weight) of 0.05 ± 0.01% (Table 1). The mean percentage for the tunica albuginea was 9.9 ± 0.4%. The volume density of seminiferous tubules and Leydig cells were 74.7 ± 3.8 and 16.7 ± 1.6%, respectively (Table 1). Therefore, Leydig cells occupied almost 70% of the intertubular compartment.

Table 1
Mean (±SEM) biometric and morphometric data regarding spermatogenesis in jaguars

End point ($n = 4$)	
Body weight (kg)	77 ± 3
Testis weight (g)	17.7 ± 2.2
Right testis	17.3 ± 2.4
Left testis	18.1 ± 2.5
Gonadosomatic index (%)	0.05 ± 0.01
Tunica albuginea weight (g)	1.7 ± 0.2
Tunica albuginea (%)	9.9 ± 0.4
Epididymis weight (g)	3.2 ± 0.4
Testis parenchyma volume density (%)	
Tubular compartment	74.7 ± 3.8
Tunica propria	4 ± 0.1
Seminiferous epithelium	61.3 ± 2.5
Lumen	9.4 ± 2.7
Intertubular compartment	25.3 ± 3.8
Leydig cell	16.7 ± 1.6
Connective tissue	0.3 ± 0.05
Blood vessels	1.1 ± 0.2
Lymphatic vessels	3.3 ± 0.4
Tubular diameter (μm)	234 ± 12
Seminiferous epithelium height (μm)	77 ± 5
Tubular length per gram of testis (m)	18.0 ± 2.7
Total tubular length per testis (m)	274 ± 36
Testis parenchyma volume (mL)	15.6 ± 2.1

Mean tubular diameter and epithelium height were 234 ± 12 and 77 ± 5 μm (Table 1). Based on the volume of the testis parenchyma (testis weight minus tunica albuginea weight), and the volume occupied by seminiferous tubules in the testis and the tubular diameter, there were 18 ± 2.7 and 274 ± 36 m of seminiferous tubules per testis gram and per testis (Table 1).

3.2. Stages of the seminiferous epithelium cycle and relative stage frequencies

3.2.1. Tubular morphology system

Based on the criteria used for determining stages (using the tubular morphology system), eight stages of the cycle were characterized (Fig. 1), as follows:

3.2.1.1. Stage 1. Only one spermatid generation was present in this stage. Spermatids had round nuclei and formed several layers within the upper part of the seminiferous epithelium. Eventual type A spermatogonia and two generations of primary spermatocytes were present; preleptotene in the transition to leptotene, with nuclei located closer to the basal lamina; and pachytene, sandwiched between round spermatids and preleptotene/leptotene spermatocytes.

3.2.1.2. Stage 2. At this stage, spermatid nuclei began elongation and the chromatin of the young elongated spermatids was more condensed than in the previous stage. Primary spermatocytes were in the transition from leptotene to zygotene, and pachytene spermatocytes nuclei were noticeably larger than in Stage 1. Type A spermatogonia were also present.

3.2.1.3. Stage 3. Elongated spermatids first formed bundles, with their heads oriented towards the Sertoli cell nuclei (usually located at the base of the tubule). Young primary spermatocytes had characteristics of zygotene cells. At the end of this stage, pachytene spermatocyte transitioned to diplotene phase of meiotic prophase. Type A spermatogonia nuclei were more frequent and similar in appearance than those in the previous stage.

3.2.1.4. Stage 4. The main feature of this stage was the presence of meiotic figures of the first and second divisions; secondary spermatocytes and early round spermatids were also observed. Zygotene spermatocytes were present and, at the end of this stage, they were in transition to pachytene spermatocytes. Elongated spermatid bundles were located within Sertoli cell crypts at approximately the middle of the seminiferous epithelium. Type A spermatogonia nuclei were present in higher numbers.

3.2.1.5. Stage 5. Two generations of spermatids were present, including early round spermatids and elongate spermatids. The young spermatid nuclei had a more heterochromatic to dusty euchromatic chromatin. Some elongate spermatid bundles were located deep within the epithelium; many were closer to the epithelium base than to the lumen. Type A spermatogonia and intermediate spermatogonia nuclei were present at the base of the tubule. Young pachytene spermatocytes were the predominant cell type, located between round spermatids and the basal lamina.

3.2.1.6. Stage 6. The onset of this stage was defined by appearance of the acrosomic vesicle in the round spermatids. The elongated spermatids bundles had moved considerably toward the seminiferous tubule lumen. Pachytene spermatocytes nuclei were bigger than in Stage 5 and more distant from the basal lamina. Type B spermatogonia were present in this stage; their nuclei were characterized by their round to ovoid shape and the

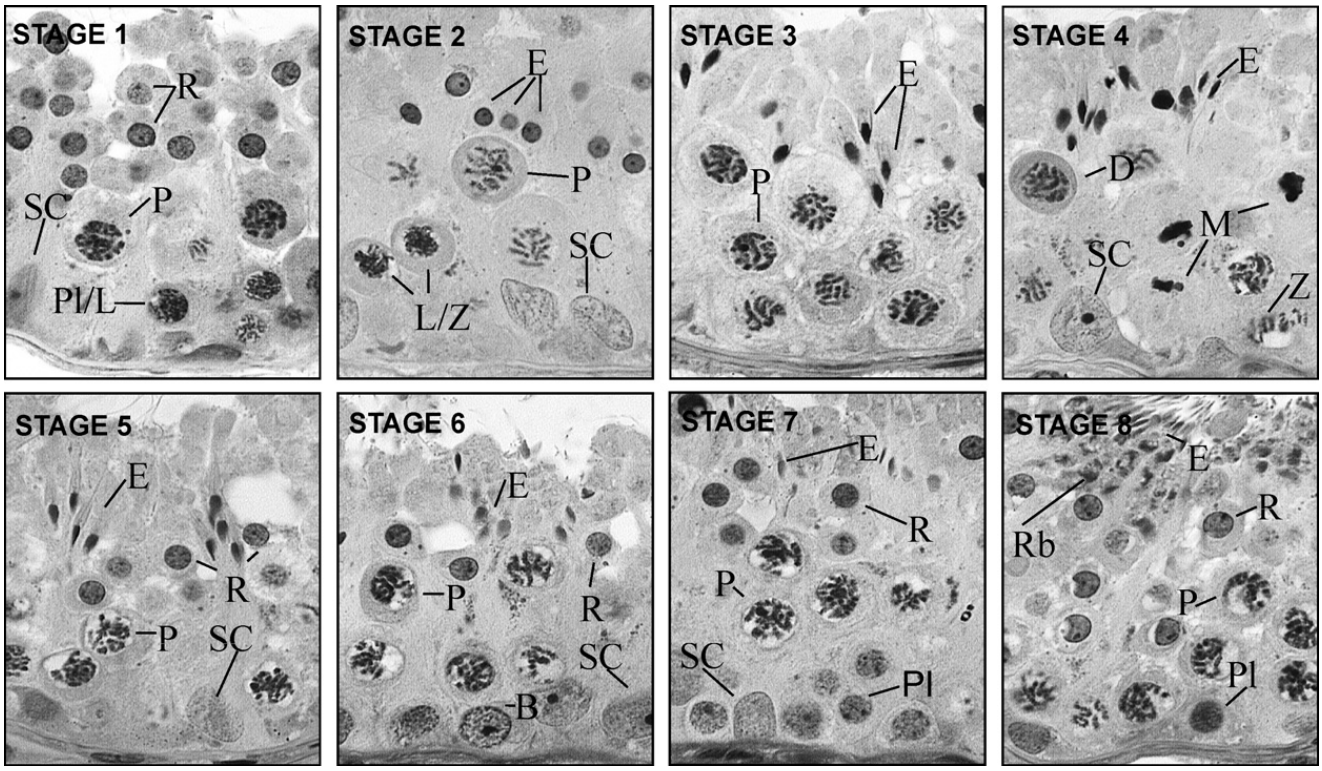


Fig. 1. Stages 1–8 of the seminiferous epithelium cycle in jaguars, based on the tubular morphology system: type B spermatogonia (B); preleptotene spermatocyte (Pl); leptotene spermatocyte (L); zygotene spermatocyte (Z); pachytene primary spermatocyte (P); diplotene spermatocyte (D); meiotic figure (M); round spermatids (R); elongating/elongate spermatids (E); Sertoli cells (SC); and residual bodies (Rb).

presence of a large amount of heterochromatin. Type A spermatogonia were eventually observed in this stage.

3.2.1.7. Stage 7. Elongated spermatid bundles had dissociated and spermatids nuclei were located very close to the tubular lumen. Type A spermatogonia, pachytene spermatocytes with bigger nuclei, round spermatids, preleptotene spermatocytes, originated from type B spermatogonia, and in contact with the basal lamina, were the other germ cell types present.

3.2.1.8. Stage 8. The main characteristic of this stage was the location of elongated spermatids just being released at the luminal aspect of the seminiferous tubule. Residual bodies were observed just below elongated spermatids. Overall, the nuclear morphology of the round spermatids, pachytene spermatocytes, preleptotene spermatocytes and type A spermatogonia present were similar to the previous stage.

Considering all eight stages characterized, the perpendicular disposition of Sertoli cell nuclei, in relation to the basement membrane, predominated in all stages of the cycle, particularly in the stages near meiotic divisions. The same trend occurred for nucleoli of Sertoli cells (mean diameter, approximately 2.5 μm).

Since a tubular cross-section could have more than one stage, frequencies of stages were based on the predominant cellular association observed. Mean percentages of each of the eight stages of the seminiferous epithelium cycle, characterized according to the tubular morphology system (Fig. 1), were as follows: Stage 1, 7.5 ± 1.1; Stage 2, 13.2 ± 2.2; Stage 3, 4.2 ± 1.1; Stage 4, 19.7 ± 1.7; Stage 5, 14.5 ± 2; Stage 6, 8.7 ± 1.5; Stage 7, 7.3 ± 1.5; and Stage 8, 25 ± 2. Therefore, Stage 8 was the most frequent, whereas Stage 3 was the least frequent. The frequencies of pre-meiotic (Stages 1–3), meiotic (Stage 4) and post-meiotic (Stages 5–8) stages were 24.9, 19.7, and 55.4%, respectively.

3.2.2. Acrosomic system

Eight stages of the cycle were also characterized according to the acrosomic system in jaguars and domestic cats, and the germ cell morphology, cell composition in each stage of the cycle, and acrosome development seemed quite similar in both species. The stages of the cycle (Fig. 2) are described below. To better characterize the stages of the cycle, we measured the angle formed by the acrosome in relation to the spermatid nucleus.

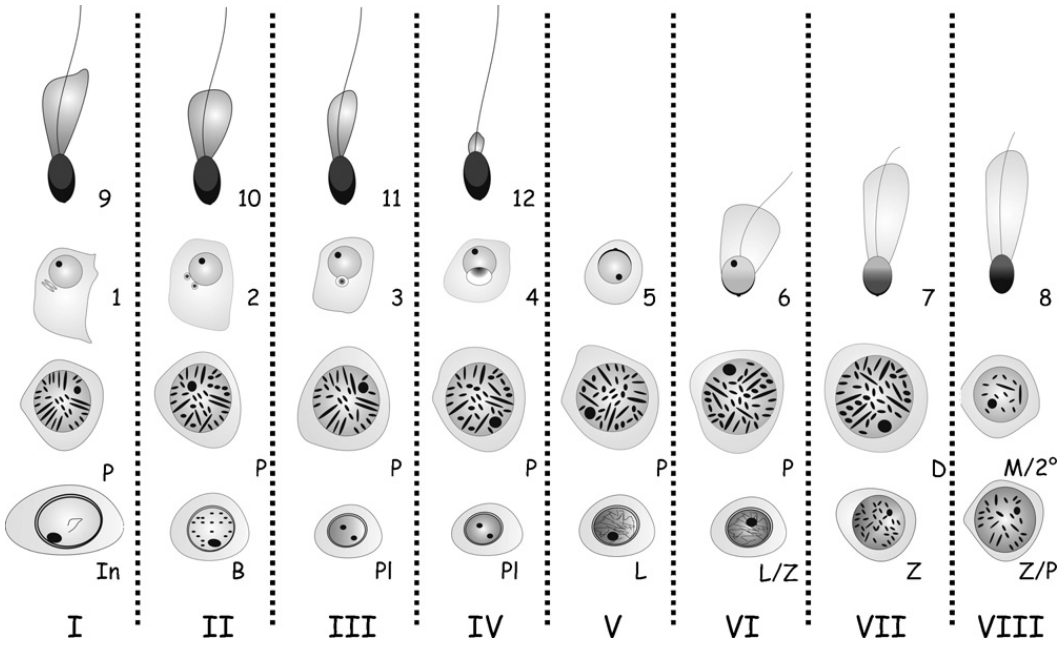


Fig. 2. This diagrammatic figure illustrates the VIII stages of the seminiferous epithelium cycle characterized in jaguars, according to the development of the acrosome in the spermatids. The vertical columns, designated by roman numerals, depict the cell associations. The developmental progression of a cell is followed horizontally until the right-hand border of the diagram is reached. The cell progression continues at the left of the diagram, one row up. The cycle diagram ends with the completion of spermiation. The following symbols were used to designate specific germ cell types: type A (A), intermediate (In), and type B (B) spermatogonia; preleptotene (Pl), leptotene (L), zygotene (Z), pachytene (P), and diplotene (D) primary spermatocyte, and secondary spermatocyte (II). Arabic numbers were used to designate the steps of the spermiogenic phase.

3.2.2.1. Stage I. Because the proacrosomal granules cannot be seen at the light microscope level, the newly-formed spermatids present in this stage were characterized by their lack of distinguishing features. However, a juxtanuclear Golgi apparatus was evident.

3.2.2.2. Stage II. Early round spermatids usually had two small acrosomal vesicles in which only occasional proacrosomal granules were present. At the end of this stage, the small proacrosomal vesicles coalesced to form one large acrosomal vesicle containing a single acrosomal granule, and the acrosomal vesicle made contact with the nucleus.

3.2.2.3. Stage III. The acrosome spread slightly over the nucleus during this stage and the acrosomal vesicle remained round. The acrosome vesicle in jaguars and in domestic cats subtended an angle on the nuclear surface of $50 \pm 3.8^\circ$ (range, ~30 to ~70) and $51 \pm 4.5^\circ$ (range, ~30 to ~70), respectively.

3.2.2.4. Stage IV. Extensive acrosomal vesicle was seen in spermatids in this stage. The acrosome vesicle extended over the nucleus and the acrosomic vesicle began to flatten where it contacted the nucleus. The acrosome vesicle subtended an angle on the nucleus of approximately $90 \pm 4^\circ$ (range, ~70 to ~110) for both species.

3.2.2.5. Stage V. In this stage, the nuclei of spermatids were still round, and the acrosome vesicle subtended over the nucleus by an angle of approximately $120 \pm 8^\circ$ (~90 to ~150) for both species.

3.2.2.6. Stage VI. The spermatid nuclei began to elongate. The ratio between the shortest axis (transverse line passing across the nucleus at the equatorial zone) and the longest or longitudinal axis was approximately 1.3 for both species.

3.2.2.7. Stage VII. Elongation of spermatids was completed during this stage. The ratio between the longest and the shortest axis of the nucleus in jaguars was 1.6 ± 0.1, whereas in domestic cats the value was 2.7 ± 0.3. Condensation of the nucleus, reflected by staining intensity, was present during the latter phase of this stage.

3.2.2.8. Stage VIII. In comparison to the previous stage, nuclei of elongate spermatids had a similar shape. Judged by staining affinity, condensation of these cells was still occurring.

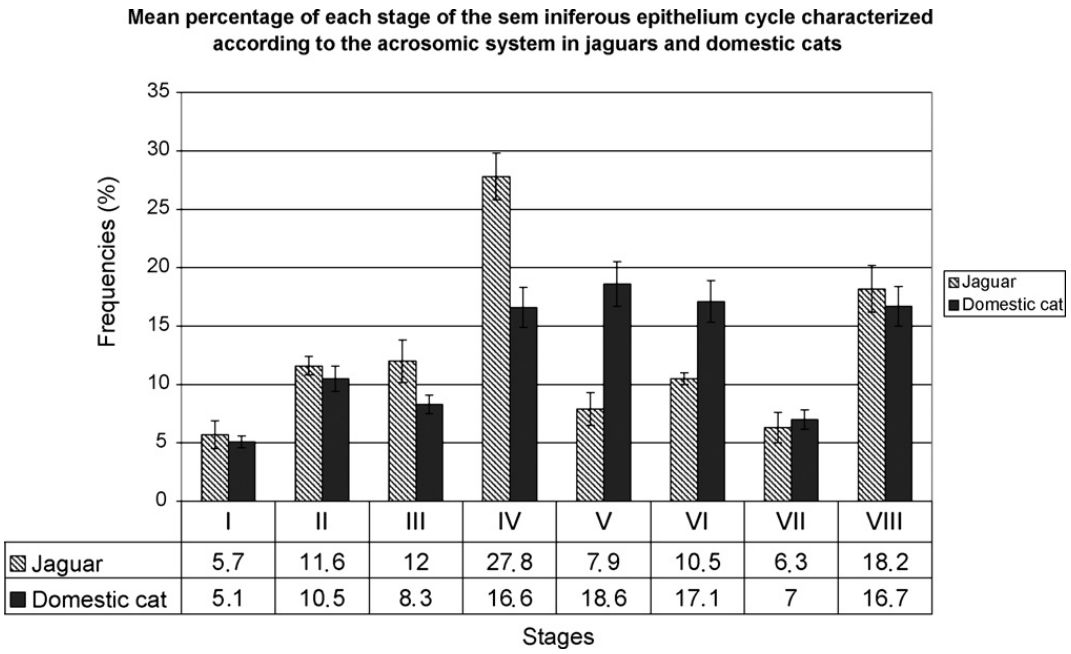


Fig. 3. Frequencies of eight stages of the cycle, characterized according to the development of the acrosome in the spermatids in jaguars ($n = 4$) and domestic cats ($n = 25$). Note that the frequencies of Stages IV, V, and VI were quite different in these two felid species and that in both species, Stages I and VII had the lowest frequencies.

The mean percentage of each of the eight stages of the seminiferous epithelium cycle, characterized according to the acrosome development for both species, are shown (Fig. 3). Except for the intermediate stages such as IV, V, and VI, most of these stages seemed to have similar frequencies. In comparison to the tubular morphology system, in jaguars the frequencies of pre-meiotic (Stages V–VII), meiotic (Stage VIII) and post-meiotic (Stages I–IV) stages of the cycle were very similar, utilizing the stages characterized according to the acrosomic system.

3.3. Length of seminiferous epithelium cycle

The most advanced labeled germ cell type observed at different time periods after thymidine injections are shown (Table 2 and Figs. 4 and 5). Approximately 1 h after injection, the most advanced labeled germ cells were identified as preleptotene spermatocytes or cells in the transition from preleptotene to leptotene. These cells were present at the end of Stage 8 and located in the basal compartment. The most advanced germ cell type labeled 18 d after thymidine injection was secondary spermatocytes at Stage 4.

Based on the most advanced labeled germ cell type observed at each time period after thymidine injections, and stage frequencies, the mean duration of the seminiferous epithelium cycle for the two animals investigated in this aspect was estimated to be 12.8 ± 0.01 d. The duration of various stages of the cycle was determined, taking into account the cycle length and the percentage of occurrence of each stage.

Table 2
Mean (±SEM) length (d) of the seminiferous epithelium cycle in jaguars

Animal	Interval after injection	Most advanced germ cell type labeled	Stage of the cycle	No. of cycles traversed	Cycle length based on labeling in leptotene
1	1 h	Pl/L[a]	8	–	–
	17.993 d[c]	M/S[b]	4	1.406	12.79
2	1 h	Pl/L[a]	8	–	–
	18.125 d[c]	M/S[b]	4	1.414	12.82
Mean duration of the cycle based on Pl/L = 12.81 ± 0.01 d.					

[a] Pl/L, preleptotene/leptotene primary spermatocytes.
[b] M, meiotic figures from first and second meiotic division; S, secondary spermatocytes.
[c] Total time after thymidine injection − 1 h.

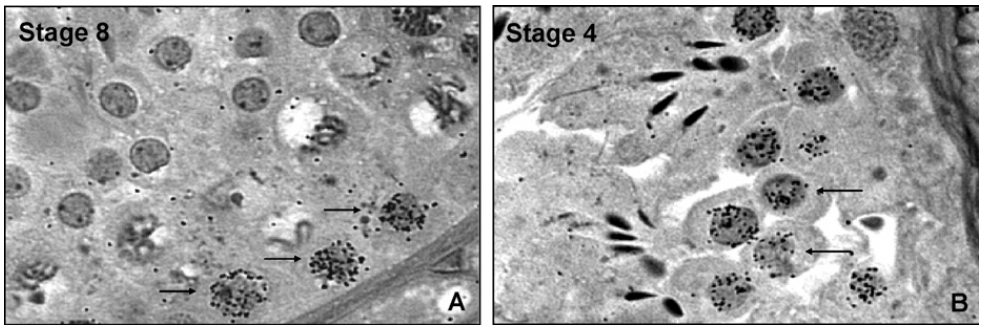
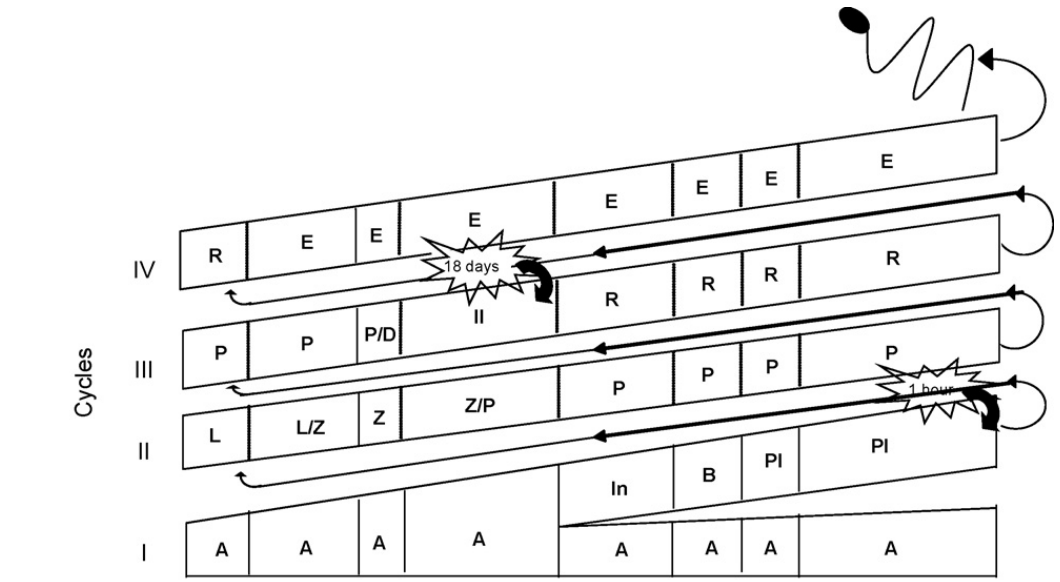


Fig. 4. The most advanced-labeled germ cells found at two intervals after intratesticular injections of tritiated thymidine in jaguars. (A) One hour after injection, preleptotene/leptotene spermatocytes (arrows) at Stage 8. (B) Eighteen days after injection, secondary spermatocytes (arrows) at Stage 4.

The shortest stage was Stage 3 (0.53 d), whereas the longest stage was Stage 8 (3.2 d). Considering that approximately 4.5 cycles are necessary for the spermatogenic process to be completed, the total length of spermatogenesis was estimated as 57.6 ± 0.07 d.

3.4. Testis morphometry

The meiotic index, measured as the number of round spermatids produced per pachytene primary spermatocytes, was 2.8 ± 0.3 (Table 3). Therefore, 30% of cell loss occurred during meiotic prophase. Sertoli cell efficiency in jaguars, estimated from the total number of germ cells and the number of round spermatids per each Sertoli cell, was 18.7 ± 2.6 and 7.9 ± 1.5, respectively (Table 3). The number of Sertoli cells per gram of testis was $29 \pm 4 \times 10^6$, whereas per testis it was $443 \pm 73 \times 10^6$ (Table 3). Regarding spermatogenic efficiency, the daily sperm production per gram of testis and per testis in jaguar was approximately 17 ± 1.2 and $262 \pm 34 \times 10^6$, respectively (Table 3).



Stages	1	2	3	4	5	6	7	8
Frequency (%)	7.5	13.2	4.2	19.7	14.5	8.7	7.3	25
Duration (days)	1	1.7	0.5	2.5	1.9	1.1	0.9	3.2

Fig. 5. Diagram showing the germ cell composition, the frequencies (%), and the duration in days of each stage of the seminiferous epithelium cycle in jaguars. Also depicted is the most advanced germ cell type labeled at the eight stages of the cycle at two intervals, 1 h and 18 d, after injection of tritiated thymidine. The Roman numerals indicate the spermatogenic cycle. The space given to each stage is proportional to its frequency and duration. The letters within each column indicate germ cell types present at each stage of the cycle. A, type A spermatogonia; In, intermediate spermatogonia; B, type B spermatogonia; Pl, preleptotene spermatocytes; L, leptotene; Z, zygotene; P, pachytene; D, diplotene; II, secondary spermatocytes; R, round spermatids; and E, elongating/elongate spermatids.

Table 3
Mean (±SEM) cell counts, cell ratios and sperm in jaguars

End point ($n = 4$)	
Round spermatids:pachytene spermatocyte	2.8 ± 0.3:1
Round spermatids:Sertoli cell nucleoli	7.9 ± 0.8:1
Sertoli cell number per gram of testis (10^6)	29 ± 4
Sertoli cell number per testis (10^6)	443 ± 73
Daily sperm production per gram of testis (10^6)	16.9 ± 1.2
Daily sperm production per testis (10^6)	262 ± 34

Table 4
Mean (±SEM) Leydig cell morphometry in jaguars

End point ($n = 4$)	
Nuclear diameter (μm)	7.4 ± 0.3
Leydig cell volume (μm^3)	1.602 ± 180
Nucleus volume (μm^3)	216 ± 31
Cytoplasm volume (μm^3)	1.386 ± 151
Leydig cell number per testis (10^9)	1.7 ± 0.2
Leydig cell number per gram of testis (10^6)	107 ± 12

For Leydig cells, nuclear volume and size were 216 ± 31 and 1602 ± 180 μm^3, respectively, whereas their number per gram of testis and per testis were 107 ± 12 × 10^6 and 1.7 ± 0.2 × 10^9 (Table 4).

4. Discussion

Our laboratory recently published a detailed quantitative and histological investigation regarding spermatogenesis and the testis in the domestic cat [20]. However, although there are some reports in the literature regarding spermatogenesis in puma and lion [25,26], to our knowledge the present investigation is the most comprehensive and detailed study investigating testis structure and function in a wild felid.

The knowledge of spermatogenic cycle length is fundamental for determining the spermatogenic efficiency (daily sperm production per testis gram) which is very useful for species comparisons [6,10,27]. For instance, in mammals 4–60 × 10^6 spermatozoa are produced daily per gram of testis parenchyma. Although species with a shorter spermatogenic cycle length have higher spermatogenic efficiency [6,10,27], in some mammals, this higher efficiency resulted from the combination of higher Sertoli cell support capacity for germ cells and a greater number of Sertoli cells per gram of testis [7,10,27,28]. The total duration of spermatogenesis in jaguar was ~25% longer than that found for the domestic cat in studies recently developed in our laboratory [20]. Also, in comparison to the domestic felid, jaguars had lower seminiferous tubule volume density (~15%). However, they had similar daily sperm production per gram of testis (16.9 × 10^6 vs 15.8 × 10^6), probably due to its higher Sertoli cell efficiency, measured as the number of spermatids per each Sertoli cell (7.9 vs 5.1) and similar number of Sertoli cells (~30 × 10^6) per testis gram [20]. Sertoli cell efficiency in jaguars was similar to that of the adult captive African lion [26].

Germ cell apoptosis occurs during spermatogenesis in all mammals investigated [7,10,27,29,30], mainly during spermatogonial phase (density-dependent regulation) as a possible homeostatic mechanism to limit germ cells to the number that can be supported by available Sertoli cells [10,31], and during meiosis, probably due to chromosomal damage [7,10,27,29]. Similar to the few felids investigated in this aspect (domestic cats and African lions) and to most mammalian species, 30% of germ cell loss occurred during meiosis in jaguars.

Compared with the mammalian species already investigated [32], the gonadosomatic index found for jaguar was very low, almost 40% smaller than that for domestic cats [20]. Similarly, the percentage occupied by the tunica albuginea in the testis represented only approximately 50% of that reported for domestic cats [20].

The relative mass of seminiferous tissue determines how much space is devoted to sperm production; in most mammals investigated, seminiferous tubules comprised the main compartment of the testis and occupied from ~70 to 95% of testis parenchyma [7,9,10]. Thus, compared with other mammalian species, the value for jaguars was in the lower part of the range, approximately 15% smaller than in domestic cats [20]. However, estimates of tubular diameter in the present study were within the range cited for most mammals investigated (180–350 μm) [29,33] and similar to that in domestic cats [20]. Regarding the intertubular compartment, in comparison to most mammals investigated, the value observed for Leydig cell size in jaguars was not low and was similar to domestic cats [7,20,34]. Conversely, Leydig cell volume density in jaguars, as well the number of Leydig cells per gram of testis, was approximately three-fold higher than in domestic cats, and within the upper range among mammalian species already investigated [7,20,34].

In the present study, eight stages were characterized according to the tubular morphology system. Also, in a comparative investigation in the present work, for both jaguar and domestic cat, eight stages were characterized

based on acrosome development. Although only four jaguars were utilized, in both criteria employed, several stage frequencies seemed to be different between these two species, even when the frequencies of these stages were grouped in pre- and post-meiotic, which according to strong evidence in the literature, might be phylogenetically determined among members of the same mammalian family [7,35]. According to molecular genetic assessment, the phylogenetic divergence between domestic cats and jaguars, which are placed in the two extremities of the Felidae family, is approximately 5×10^6 years [1]. Stage frequencies in jaguar were also quite different from those found in puma [25]. However, the latter were obtained from testis biopsy, which provided a small area for this evaluation. It should be mentioned that different from the domestic cat [20,36], sperm pleiomorphisms and missing generations of germ cells were not observed in jaguars. Unfortunately, we did not find a clear morphological marker to distinguish germ cells derived from jaguars versus domestic cats.

The loss of genetic diversity is a serious threat to the conservation of endangered species, including wild felids [37]. However, several assisted reproductive techniques and biotechnologies could be used to propagate small, fragmented populations of wild endangered species [38,39]. Among these possibilities, germ cell transplantation can be used to introduce male germ cells from one animal into another of the same [40] or a different species [34,41], resulting in the growth and differentiation of germ cells. Furthermore, germ cells can be frozen [42] and cultured [13] before transplantation. We are currently developing spermatogonial stem cell transplantation techniques in the domestic cat as a tool to preserve and propagate male germ plasm from wild endangered felid species.

In conclusion, in the present study, we obtained for the first time, comprehensive basic data related to testis function in jaguars, including characterization of the cycle of the seminiferous epithelium, the duration of spermatogenesis, and the Sertoli cells, and spermatogenic efficiencies. We are optimistic that these data will provide the necessary background for future research involving germ cells transplantation from jaguars to the domestic cats and, consequently, facilitate preservation of the genetic stock from jaguars.

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