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Duration of spermatogenesis and daily sperm production in the rodent *Proechimys guyannensis*

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Summary

The spiny rat (*Proechimys guyannensis*) is a neotropical rodent that is used in biomedical research, particularly research related to chronic resistance to epilepsy and infectious diseases. To our knowledge, there are few reports concerning the reproductive biology of this species. Therefore, besides providing basic biometric and morphometric data, in the present study we investigated testis function and spermatogenesis in adult spiny rats. The mean testis weight and gonadosomatic index obtained were 1.63 ± 0.2 g and 1.15 ± 0.1% respectively. Based on the development of the acrosomic system, 12 stages of the seminiferous epithelium cycle were characterized. Stages VI and VII presented the highest frequencies (~17–19%), whilst stages II to V showed the lowest frequencies (~2–4%). The most advanced germ cell types labelled at 1 h or 20 days after BrdU injections were respectively preleptotene/leptotene spermatocytes at stage VII and elongated spermatids at stage III. The mean duration of one cycle was 7.5 ± 0.01 days and the entire spermatogenic process lasted 33.7 ± 0.06 days (~4.5 cycles). The seminiferous tubules (ST) occupied ~96 ± 1% of the testis parenchyma, whereas Leydig cells comprised only 1.5 ± 0.4%. The number of Sertoli cells (SC) per testis gram and the SC efficiency (spermatids/SC) were respectively $78 \times 10^6 \pm 11 \times 10^6$ and 7.9 ± 1. The daily sperm production per testis gram (spermatogenic efficiency; daily sperm production (DSP)/g/testis) was $78 \times 10^6 \pm 8 \times 10^6$. To our knowledge, this spermatogenic efficiency is among the highest found for mammals investigated to date and is probably related to the very short duration of spermatogenesis and the very high ST percentage and SC number obtained for this species.

Keywords: *P. guyannensis*, Sertoli cell efficiency, Sperm production, Spermatogenesis, Spiny rat, Testis

Introduction

The order Rodentia is the largest from the Mammalia class, representing about 40% of the mammalian species alive at present, with approximately 29 families, 443 genera and more than 2000 species that occur in almost all habitats (Nowak, 1999; Lange & Schmidt, 2007). The genus *Proechimys* (sub-order Hystricomorpha, family Echimyidae) represents one of the most diverse groups of neotropical rodents, with 25 species (Wilson & Reeder, 2005). These rodents are terrestrial, nocturnal and called 'spiny rat' because of their rigid and prickly hair. Species from this genus are frugivorous but they also eat seeds and

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fungi (Eisenberg & Redford, 1999; Catzeflis & Patton, 2008), and reproduces throughout the year (Weir, 1973; Nowak, 1999; Patton *et al*, 2000).

The *Proechimys guyannensis* was described by Geoffroy (1803) and was successfully raised in the laboratory, being used for experimental research since 1960 (Hawking *et al.*, 1964). A remarkable characteristic of this species is the high degree of development of the newborns (Arida *et al.*, 2005; Scorza *et al.*, 2011). Due to its resistance to chronic epilepsy, this species has been used as a model for this disease (Cavalheiro, 1995; Fabene *et al.*, 2001; Arida *et al.*, 2005; Rocha *et al.*, 2006; Silva *et al.*, 2014), being also, as a natural host, used in studies involving infectious parasites (Everard & Tikasingh, 1973). Investigations related to ecology and evolution of *P. guyannensis* are also available in the literature (Steiner *et al.*, 2000; Eler *et al.*, 2012; Silva *et al.*, 2012).

Although testis structure and organization can be very similar between mammals, each species may exhibit particular morphofunctional characteristics, such as those related to phylogenetic aspects and reproductive strategy/behaviour (Kerr *et al*, 2006; Setchell & Breed, 2006). Therefore, knowledge of male reproductive biology and physiology is fundamental for comparative and evolutionary studies (Wildt, 2005). This knowledge is also important to prevent species from extinction, as well as to improve species management and enhance male reproductive capacity in natural and artificial breeding programmes (Comizzoli *et al*, 2000). The *P. guyannensis* reproduces throughout the year and its estimated age of puberty is around 83 days old (Tesh, 1970; Weir, 1973; Nowak, 1999; Patton *et al*, 2000; Madureira *et al.*, 2014); however, its reproductive biology is poorly known. Therefore, the aims of the present study were to perform a detailed histological and stereological/morphometric analysis of testis structure and function, which allowed the estimation of key reproductive parameters such as duration of spermatogenesis and the SC and spermatogenic efficiencies for this species.

Materials and methods

Animals

Ten sexually mature (180 days old) males *Proechimys guyannensis* were used in the present study. The animals came from the vivarium of the Federal University of São João del-Rei (UFSJ), in Minas Gerais, Brazil. All procedures and protocols followed approved guidelines for the ethical treatment of animals (CETEA/UFMG).

BrdU injections and tissue preparation

To estimate the duration of spermatogenesis, two animals received intraperitoneal injections of 5-bromo-2′-deoxyuridine (BrdU; 150 mg/kg), a specific marker for cells that are synthesizing DNA. Two time intervals (1 h and 20 days) following BrdU injections were considered for each animal. At sacrifice, all 10 animals received an i.p. injection of sodium thiopental (50 mg/kg), and testes were removed, weighed and fixed by immersion in buffered glutaraldehyde or Bouin's fixative. Then, testes were cut transversally, routinely processed and embedded in plastic (glycol methacrylate) for histomorphometric analyses or in paraplast for BrdU analyses.

Immunostaining for BrdU

Immunohistochemical staining of cells in S-phase using anti-BrdU antibody was performed as briefly follows. Sections 5 μm thick were mounted on coated slides, dewaxed and rehydrated. Then, antigen retrieval was performed in citrate buffer (pH 6.0) for 5 min after boiling in a microwave oven. Slides were incubated in 30% hydrogen peroxide (Sigma Aldrich) for 30 min at room temperature, to block endogenous peroxidase activity. Nonspecific binding sites were blocked with 10% normal horse serum (VectorStain ABC kit, Vector Laboratories) in PBS before the addition of primary antibody anti-BrdU (BD Biosciences, R2232, 1:200) and incubated overnight at 4°C. After this procedure, the slides were exposed to horse anti-mouse secondary antibody (1:200, VectorStain ABC kit, Vector Laboratories) for 60 min at room temperature. Detection of the signal was performed by incubating the sections in streptavidin (Thermo Scientific, TS-125-HR) for 30 min at room temperature, followed by the reaction with peroxidase substrate 3,3′-diaminobenzidine (DAB, Sigma Aldrich) and counterstaining with hematoxylin (Merck). Following dehydration, sections were mounted and analyzed by light microscopy to detect the most advanced germ cell type labelled at the two different time intervals evaluated after BrdU injection.

Testis morphometry

Sections of 4-μm thickness obtained from plastic embedded testis were stained with toluidine blue. The volume densities of the testicular components were determined on images captured by light microscopy, using a 540-intersection grid from ImageJ software (National Institutes of Health, http://rsb.info.nih.gov/ij/). Fifteen randomly chosen fields/images (8100 points) were scored for each animal ($n = 6$) at ×400 magnification. Points were classified as one of the following: seminiferous

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epithelium, tunica propria, lumen, Leydig cell, connective tissue, blood or lymphatic vessels. The volume of each testis component was determined as the product of its volume density and testis parenchyma volume. For subsequent morphometric calculations, the specific density of testis tissue was considered to be 1.0 (França & Godinho, 2003; Leal & França, 2006). To obtain a more precise measure of testis volume, the mean value of testis capsule (3.2%) was excluded from the testis weight. Seminiferous tubular diameter and height of the seminiferous tubule epithelium were measured at ×400 magnification using an ocular calibrated with a stage micrometer. Thirty round or nearly round tubular profiles were randomly chosen and measured for each animal. The total length of the seminiferous tubule (in metres) was obtained by dividing the seminiferous tubule volume by the square radius of the tubule multiplied by π (Johnson & Neaves, 1981; França & Godinho, 2003; Auharek *et al.*, 2011).

Stages and length of the seminiferous epithelium cycle

Stages of the seminiferous epithelium cycle were characterized ($n = 8$) based on the development of the acrosomic system (Russell *et al.*, 1990) and morphology of the spermatid nucleus. The relative stage frequencies were determined from the analysis of at least 200 seminiferous tubule cross-sections per animal, at ×1000 magnification, as described by Leal & França (2006). The histological sections used were those that presented high quality and more tubular cross-sections.

The duration of the spermatogenic cycle was estimated based on the stage frequencies and the most advanced germ cell type labelled at the two time periods used post-BrdU 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 (Amann & Schanbacher, 1983; França & Russell, 1998; Leal & França, 2006; Hess & França, 2007). Because the nuclear volume of pachytene primary spermatocytes grows markedly during meiotic prophase (França & Russell, 1998; Neves *et al*, 2002), the size of their nuclei was used to determine more precisely the location of the most advanced labelled germ cell, particularly when these cells were present in stages showing high frequency.

Cell counts and cell numbers

All germ cells nuclei and SCs nucleoli present at stage VI (near spermiation) of the cycle were counted in ten randomly chosen round or nearly round seminiferous tubule cross-sections for each animal. These counts were corrected for section thickness (4 μm) and nuclear or nucleolar diameter according to Abercrombie (1946), as modified by Amann & Almquist (1962). For this purpose, 10 nuclei or nucleoli diameter were measured per animal for each cell type analyzed. Cell ratios were obtained from the corrected counts obtained. The total number of SCs was determined from the corrected counts of Sertoli cell nucleoli per seminiferous tubule cross-sections and the total length of ST (Hochereau-de Reviers & Lincoln, 1978; Leal & França, 2006; Costa *et al.*, 2010). Daily sperm production (DSP) per testis and per gram of testis was determined based on the formula:

$$\begin{aligned}\text{DSP} = {} & \text{total number of SCs per testis} \\ & \times \text{ratio of round spermatids to Sertoli cells at stage VI} \\ & \times \text{stage VI relative frequency (\%)/stage VI duration (days)}\end{aligned}$$

(Leal & França, 2006; Auharek *et al.*, 2011, Cordeiro-Junior *et al.*, 2010).

The individual volume of Leydig cells was obtained from the nucleus volume and the proportion between the nucleus and cytoplasm (Leal & França, 2006; Auharek *et al.*, 2011). As the Leydig cell nucleus in this species is spherical, nucleus volume was calculated from the mean nucleus diameter and, for this purpose, 30 nuclei with an evident nucleolus was measured per animal. Leydig cell nuclear volume was expressed in μm^3 and obtained by the formula $4/3\pi r^3$, where r = nuclear diameter/2. To calculate the proportion between nucleus and cytoplasm, a 441-point square lattice was placed over the sectioned material at ×1000 magnification and 1000 points over Leydig cells were counted for each animal. Subsequently, the total number of Leydig cells per testis was estimated from the Leydig cell individual volume and the volume density occupied by Leydig cells in the testis parenchyma.

Results

Biometric data and testis stereology

Biometric and testis morphometric data are shown in Table 1. The obtained testis weight was 1.63 ± 0.2 g, while the gonadosomatic index (GSI; testes mass divided by body weight) was 1.15 ± 0.2%. The ST occupied nearly 96% of the testis parenchyma, whereas Leydig cells comprised 1.5 ± 0.4%. Mean seminiferous tubule diameter and epithelium height were 168 ± 7 and 65 ± 2 μm, respectively. Based on the volume of testis parenchyma and the volume occupied by ST in the testis and the tubular diameter, the total length of seminiferous tubules per testis was 66 ± 8 m.

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Table 1 Biometric and morphometric data in *P. guyannensis*

Parameters	Mean ± SEM
Body weight (g)	288 ± 12
Testis weight (g)	1.63 ± 0.2
Gonadosomatic index (%)	1.15 ± 0.2
Tunica albuginea (%)	3.2 ± 0.3
Volume density (%)	
Tubular compartment	95.8 ± 1
Tunica propria	4.6 ± 0.5
Seminiferous epithelium	87.3 ± 2
Lumen	3.9 ± 0.7
Intertubular compartment	4.2 ± 1.1
Leydig cell	1.5 ± 0.4
Blood vessels	1.2 ± 0.4
Lymphatic space	1.0 ± 0.3
Others	0.5 ± 0.1
Tubular diameter (μm)	168 ± 7
Seminiferous epithelium height (μm)	65 ± 2
Length of seminiferous tubule per gram of testis (m)	44 ± 4
Total length of seminiferous tubule per testis (m)	66 ± 8

Stages of the seminiferous epithelium cycle and relative frequencies

Based on the development of the acrosomic system and the morphology of the developing spermatid nucleus, 12 stages of the seminiferous epithelium cycle were characterized. These stages (shown in Fig. 1) are briefly described below.

Stage I
Two generations of spermatids were observed in this stage: early round spermatids and elongated spermatids. The newly formed round spermatids were smaller than secondary spermatocytes and were characterized by the lack of proacrosomal granules, although a juxtanuclear Golgi apparatus was evident at ×1000 magnification. Elongated spermatid bundles are more packed. As occurs with all spermatogonial cells, type A differentiated spermatogonia are in contact with the basal membrane.

Stage II
Early round spermatids with two small proacrosomal vesicles were present. At the end of this stage, these proacrosomal vesicles have coalesced to form a single acrosomal vesicle in contact with the nucleus and containing an acrosomal granule. Intermediate spermatogonia are also present in the basal compartment.

Stage III
This stage begins as the acrosomal vesicle flattens over the surface of the nucleus. The elongated spermatids moved toward the seminiferous tubule lumen. Type B spermatogonia were also observed.

Stage IV
The acrosome spreads over the surface of the nucleus. The elongated spermatids bundles moved deep inside the seminiferous epithelium. As observed in the previous stage, type B spermatogonia were in contact with the basement membrane.

Stage V
Elongated spermatid bundles were more dissociated and moved toward the lumen. Type B spermatogonia in transition to preleptotene spermatocyte were present at the base of the seminiferous epithelium.

Stage VI
Elongated spermatids with a spatulated head were being released/spermiated toward the tubule lumen. Two generations of primary spermatocytes were present: preleptotene spermatocytes lining the basal membrane; and pachytene spermatocytes positioned between round spermatids and preleptotene spermatocytes.

Stage VII
Only one spermatid generation, with round nuclei and forming several layers within the upper part of the seminiferous epithelium, was present in this stage. The acrosome continued spreading over the nucleus. Preleptotene spermatocytes were in contact with the basement membrane.

Stage VIII
Together with the acrosome, the spermatid nuclei begun to elongate, becoming ovoid in shape.

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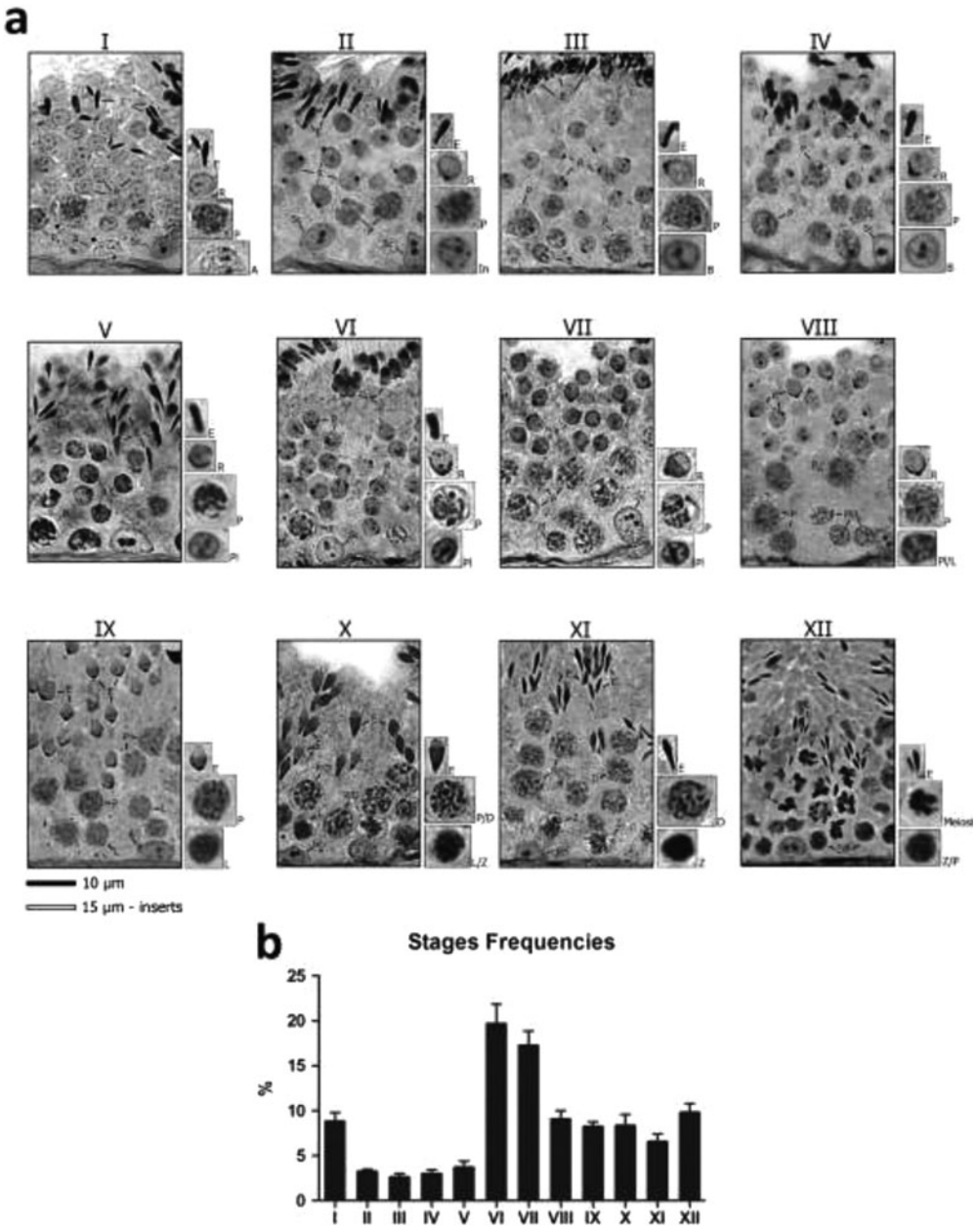

Figure 1 Stages of the seminiferous epithelium cycle and its frequencies. (*a)* Stages I–XII of the seminiferous epithelium cycle in *P. guyannensis* based on the acrosomic system. The individual germ cell nuclei shown in the right column represent the germ cells found in each particular stage. A: type A differentiated spermatogonia; In: Intermediate spermatogonia; B: type B spermatogonia; Pl: preleptotene spermatocytes; L: leptotene spermatocytes; Z: zygotene spermatocytes; P: pachytene spermatocytes; D: diplotene spermatocytes; Meiosis: meiotic figures; R: round spermatids; E: elongating/elongated spermatids; SC: Sertoli cells. (*b*) Frequencies (mean percentage ± SEM) of the 12 stages of the cycle in *P. guyannensis.*

Preleptotene spermatocytes in transition to leptotene were observed.

Stage IX

The acrosome followed the nuclear elongation of the spermatids. Elongated spermatids were with their heads oriented toward the SC nuclei. Leptotene spermatocytes were present in the base of the seminiferous epithelium.

Stage X

A ventral angle was formed in the elongated heads of the spermatids. These elongated spermatids formed bundles in the seminiferous epithelium. The pachytene

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spermatocytes were in transition to the diplotene phase of the meiotic prophase, while the leptotene spermatocytes changed to zygotene.

Stage XI
Elongation of spermatids was complete at this stage. Two generations of primary spermatocytes were present: zygotene and diplotene.

Stage XII
The presence of meiotic figures related to the first and second meiotic divisions was the main characteristic of this stage. Secondary spermatocytes and newly formed round spermatids were also observed. The zygotene spermatocytes were in the transition to pachytene.

Sertoli cells and undifferentiated type A spermatogonia were observed in all twelve stages characterized. The mean frequencies of the stages of the seminiferous epithelium cycle are displayed in Fig. 1. Stages VI and VII had the highest frequencies (19.6 and 17.2% respectively), whereas stages II–IV were less frequent (~2 to ~4%). The frequencies of pre-meiotic (stages VII–XI), meiotic (stage XII) and post-meiotic (stages I–VI) phases of the cycle were ~49, ~10 and ~41%, respectively.

Length of the seminiferous epithelium cycle

The most advanced labelled germ cell types observed following BrdU injection are shown in Fig. 2. Approximately 1 h after injection, the most advanced germ cells labelled were identified as preleptotene spermatocytes or cells in the transition from preleptotene to leptotene spermatocytes. Based on the mean pachytene nucleus diameter in both animals investigated in this aspect, these spermatocytes were present at the middle part of stage VII. At 20 days after BrdU injection, the most advanced germ cells labelled were elongated spermatids present in stage III. Based on the most advanced germ cell labelled at each time period and the stage frequencies, the mean duration of one seminiferous epithelium cycle was estimated to be 7.48 ± 0.01 days, whereas the total duration of spermatogenesis, considering that 4.5 cycles are necessary for the spermatogenic process to be completed, was estimated to be 33.6 ± 0.06 days.

Cell counts and cell numbers

The cell counts, ratios and sperm production are shown in Table 2. The meiotic index (number of round spermatids produced per primary pachytene spermatocyte) was 2.7 ± 0.1. Sertoli cell efficiency (number of round spermatids per Sertoli cell) was approximately 8 ± 1. The number of SCs per testis and per gram of testis were 112 × 10^6 ± 12 × 10^6 and 78 × 10^6 ± 11 × 10^6 cells, respectively. The DSP per testis and per gram of testis (spermatogenic efficiency) was approximately 121 × 10^6 ± 24 × 10^6 and 78 × 10^6 ± 9 × 10^6, respectively. It means that, in total, around 240 × 10^6 sperm were produced daily.

Leydig cell nuclear volume and Leydig cell individual size were 183 ± 4 and 746 ± 51 μm³, whereas their number per testis and per gram of testis were respectively 28.3 × 10^6 ± 6.7 × 10^6 and 19 × 10^6 ± 4.6 × 10^6 cells (Table 3).

Discussion

The *Proechimys guyannensis* is a neotropical rodent that is a natural host of infectious parasites and is currently used in biomedical research, particularly due to its resistance to epilepsy. Comprehensive studies on testicular structure and function have been performed on less than 2% of living mammalian species (Nowak, 1999; Almeida *et al.*, 2006; Leal & França, 2009; Cordeiro-Junior *et al.*, 2010; Costa *et al.*, 2010), and to our knowledge, there are few reports related to the reproductive biology of this species. Due to the very high ST volume density (%) and number of SCs per testis gram and the very short duration of spermatogenesis, the spermatogenic efficiency (DSP per gram of testis) obtained for *P. guyannensis* is one of the highest obtained for the mammalian species investigated to date. This DSP is similar to that observed for *Trinomys moojeni* (Cordeiro-Junior *et al.*, 2010), which is another species of the same family herein investigated. Because there are very few data available for the members of this family, the discussion below will be focused mainly on the data available for the main investigated laboratory rodents (rats, mice and hamsters) and *T. moojeni* (please see Table 4).

Based on the development of the acrosomic system, 12 stages of the seminiferous epithelium cycle were characterized in *P. guyannensis* and only one stage per seminiferous tubule cross-section was observed. This segmental arrangement of the stages is peculiar of rodents and is present in most mammals so far investigated (Leblond & Clermont, 1952; Sharpe, 1994; França *et al.*, 2005). Several studies developed in our laboratory indicates that pre-meiotic and post-meiotic stage frequencies are phylogenetically determined (França & Russell, 1998; Neves *et al.*, 2002; França *et al.*, 2005; Almeida *et al.*, 2006; Costa *et al.*, 2008; Silva *et al.*, 2010). In these studies, two clear patterns are observed for these frequencies in rodents: species in which the pre-meiotic frequency is about one-quarter of the entire spermatogenic cycle and species that show an equilibrium between the combined pre-meiotic and post-meiotic frequencies. In this aspect, similar to

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Table 2 Cell counts, cell ratios and sperm production

Parameters	Mean ± SEM
Meiotic index	2.7 ± 0.1
Sertoli cell efficiency	7.9 ± 1
Sertoli cell number per gram of testis ($\times 10^6$)	78 ± 11
Sertoli cell number per testis ($\times 10^6$)	112 ± 12
Daily sperm production per gram of testis ($\times 10^6$)	78 ± 8
Daily sperm production per testis ($\times 10^6$)	121 ± 24

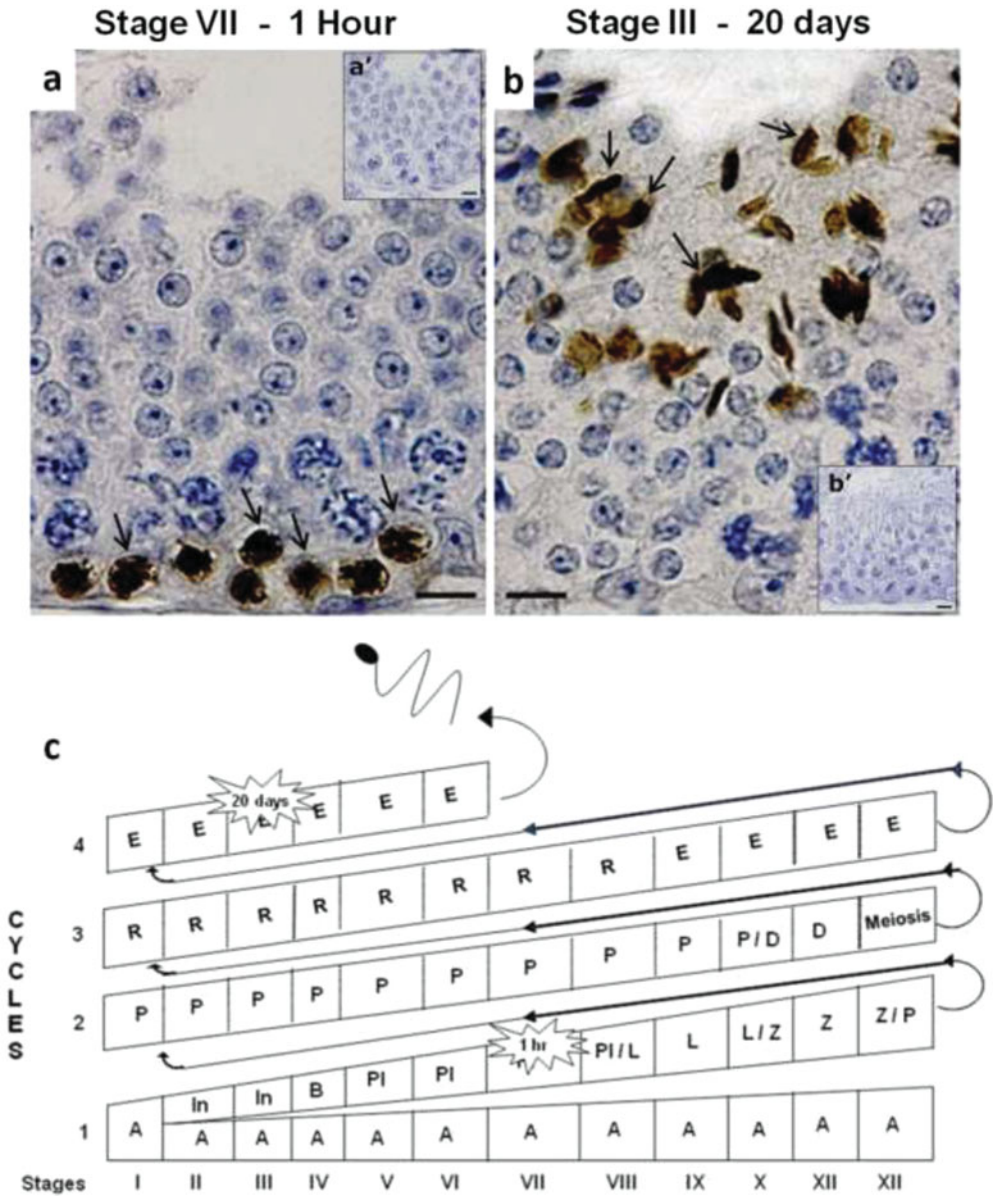

Figure 2 Most advanced germ cells labelled after BrdU injection and the germ cell composition of each stage of the seminiferous epithelium cycle. (*a*) One hour after injection, preleptotene/leptotene spermatocytes (arrows) at the middle part of stage VII are labelled. (*a*′) Negative control. (*b*) Twenty days after injection, elongated spermatids (arrows) at the stage III are labelled. (*b*′) Negative control. (*c*) Diagram showing germ cell composition of each stage of the seminiferous epithelium cycle in *P. guyannensis*. Letters within each column indicate the germ cell type present in each stage. A: type A spermatogonia; In: intermediate spermatogonia; B: type B spermatogonia; Pl: preleptotene; L: leptotene; Z: zygotene; P: pachytene; D: diplotene; R: round spermatids; and E: elongating/elongated spermatid. Scale bars: 10 μm

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Table 3 Leydig cell morphometry

Parameters	Mean ± SEM
Nuclear diameter (μm)	7 ± 0.1
Leydig cell volume (μm³)	746 ± 51
Nuclear volume (μm³)	183 ± 4
Cytoplasmatic volume (μm³)	563 ± 52
Leydig cell number per gram of testis ($\times 10^6$)	19 ± 4.6
Leydig cell number per testis ($\times 10^6$)	28.3 ± 6.7

Table 4 Comparative parameters related to biometry, testis stereology and spermatogenesis in some well investigated rodent species

Parameters	*P. guyannensis*	*T. moojeni*[a]	Mouse[b]	Rat[c]	Hamster[d]
Body weight (g)	288	207	26–39	414	160
Testis weight (g)	1.63	0.97	0.095–0.113	1.57	1.7
Gonadosomatic index (%)	1.15	0.93	0.55–0.76	0.76	2.13
Seminiferous tubules (%)	95.8	97.6	91–93	89	93
Leydig cell (%)	1.5	0.3	3.7–5.3	1.4	2.7
Leydig cell size (μm³)	746	799	1021–1450	1207	1092
Leydig cell number per gram of testis ($\times 10^6$)	19	3.8	29–49	12.6	55
Pre-meiotic phase[e] (%)	49	41	22	24	25.8
Meiotic phase[f] (%)	10	7	9	6	7.5
Post-meiotic phase[g] (%)	41	53	69	71	66.7
Meiotic index[h]	2.7 (32.5%)	3 (25%)	2.3–2.8 (43–30%)	3.4 (15%)	3.3 (17%)
Spermatogenic cycle length (days)	7.5	8.6	8.6–8.9	12.9	8.7
Total duration of spermatogenesis (days)	33.6	38.5	38.7–40	58	39.2
Sertoli cells per gram of testis ($\times 10^6$)	78	53	39–41	27	44.5
Round spermatids per Sertoli cell (SC efficiency)	7.9	14.7	10.5–11.5	8.0–10.3	8.2
Daily sperm production per gram of testis ($\times 10^6$)	78	82	45–48	17–24	24

[a]Cordeiro-Junior *et al.*, 2010.
[b]Clermont & Trott, 1969; Avelar *et al.*, 2004; Auharek *et al.*, 2011.
[c]Clermont & Harvey, 1965; Rocha *et al.*, 1999; França, 2007.
[d]Sinha Hikim *et al.*, 1988; Van Haaster & De Rooij, 1993.
[e]Combined stages frequencies after spermiation and prior to metaphase.
[f]Meiotic division I through meiosis II.
[g]Combined stages frequencies after completion of meiosis until spermiation.
[h]Measured as the number of round spermatids produced per pachytene primary spermatocyte (presumptive germ cell loss during meiosis in parenthesis).

other rodents phylogenetically closely related (Paula *et al.*, 1999; Leal & França, 2009; Costa *et al.*, 2010), *P. guyannensis* and *T. moojeni* belong to the latter pattern, whereas mice, rats and hamsters belong to the first pattern (Table 4). Conversely, the duration of spermatogenesis is considered to be species specific (Clermont, 1972; Amann & Schanbacher, 1983) and controlled by the germ cell genotype (França *et al.*, 1998). Particularly when compared with other rodent species investigated (Russell *et al*, 1990; França & Russell, 1998; França *et al.*, 2005; Hess & França, 2007; Costa *et al.*, 2010), the duration of one spermatogenic cycle (7.5 days) and the total duration of spermatogenesis (33.6 days) found in the present study is one of the shortest so far obtained.

In the literature, it is considered that total number of SCs per testis is established before puberty (Orth *et al*, 1988; Sharpe *et al*, 2003; França *et al*, 2005; Holsberger & Cooke, 2005) and that the number of this key somatic cell per seminiferous tubule cross-section is stable along the different stages of the seminiferous epithelium cycle. Therefore, these cells are used as a reference to quantify and evaluate spermatogenesis (França & Russell, 1998; Johnson *et al*, 2000; França & Hess, 2005). Specifically, the number of germ cells supported by each SC (Sertoli cell efficiency) is considered species specific and positively correlates with spermatogenic efficiency (França & Russell, 1998; Johnson *et al*, 2000; França & Hess, 2005; França *et al*, 2005; Hess & França, 2007). Although not very different from the values observed for mice, rats

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and hamsters (Sinha-Hikim *et al*, 1988; Van Haaster & De Rooij, 1993; França & Russell, 1998; Johnson *et al*, 2000; França & Hess, 2005; França *et al*, 2005; França, 2007), the result found for SC efficiency in *P. guyannensis* (7.9) is much lower than that observed (~15) for the other investigated species from the Echimyidae family (*T. moojeni*; Cordeiro-Junior *et al*., 2010). However, because the number of SCs per testis gram obtained in the present study is strikingly high and the duration of spermatogenesis is ~15% faster, a similar spermatogenic efficiency was observed for *P. guyannensis* when compared to *T. moojeni* (Cordeiro-Junior *et al*., 2010). For most mammalian species already investigated, including laboratory rodents such as mice, rats and hamsters, this efficiency ranges from 20×10^6 to 50×10^6 (Sharpe, 1994; Johnson *et al*., 2000; Hess & França, 2007; Sousa *et al*., 2014). Therefore, the spermatogenic efficiency found for *P. guyannensis* can be included in the top level for mammals (Table 4).

In comparison with most mammalian species investigated (Kenagy & Trombulak, 1986; França, 2007; Leal & França, 2008; Cordeiro-Junior *et al*., 2010; Costa *et al*., 2010), the gonadosomatic index (GSI) in *P. guyannensis* is relatively high. In the literature, it is considered that species with higher GSI are usually promiscuous (Kenagy & Trombulak, 1986; Short, 1997; Costa *et al*., 2010). Besides that, only two to three pups are observed per gestation in this species (Madureira *et al*., 2014). These aspects deserve further investigation in *P. guyannensis*. Because the ST volume density observed in the present study is strikingly high (~96%), it could be expect that the Leydig cell volume occupancy would be very low. However, due to the relatively small cell size, the obtained number of this steroidogenic cell per testis gram is similar to many rodents so far investigated, such as agouti and paca (Costa *et al*., 2010) and rats (Clermont & Harvey, 1965; Rocha *et al*, 1999; França, 2007), and even higher than the values found for *T. moojeni* (Table 4).

In summary, due to the very high seminiferous tubule volume density and number of SCs per testis gram, as well as to the short duration of spermatogenesis, the spermatogenic efficiency found in the present study for *P. guyannensis* is strikingly high. However, surprisingly, the number of pups per litter cited in the literature for this rodent is very small. Therefore, besides its importance in biomedical research, this species may represent an interesting model for investigating reproductive strategies in mammals.

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Declaration of conflicting interests

None.

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