Zygote **25** (August), pp. 498–506. © Cambridge University Press 2017
doi:10.1017/S0967199417000326 First Published Online 11 July 2017

Morphofunctional evaluation of the testis, duration of spermatogenesis and spermatogenic efficiency in the Japanese fancy mouse (*Mus musculus molossinus*)

Guilherme M.J. Costa[2], *Marcelo C. Leal*[3] *and Luiz R. França*[1,4]
Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte; UFLA, Animal Science, Lavras; and INPA, Manaus, Brazil

Date submitted: 15.03.2017. Date revised: 16.05.2017. Date accepted: 18.05.2017

Summary

Japanese fancy mouse, mini mouse or pet mouse are common names used to refer to strains of mice that present with different colour varieties and coat types. Although many genetic studies that involve spotting phenotype based on the coat have been performed in these mice, there are no reports of quantitative data in the literature regarding testis structure and spermatogenic efficiency. Hence, in this study we researched testis function and spermatogenesis in the adult Japanese fancy mouse. The following values of 68 ± 6 mg and 0.94 ± 0.1% were obtained as mean testis weight and gonadosomatic index, respectively. In comparison with other investigated mice strains, the fancy mouse Leydig cell individual size was much smaller, resulting in higher numbers of these cells per gram of testis. As found for laboratory mice strains, as a result of the development of the acrosomic system, 12 stages of the seminiferous epithelium cycle have been described in this study. The combined frequencies of pre-meiotic and post-meiotic stages were respectively 24% and 64% and very similar to the laboratory mice. The more differentiated germ cell types marked at 1 h or 9 days after tritiated thymidine administration were preleptotene/leptotene and pachytene spermatocytes at the same stage (VIII). The mean duration of one spermatogenic cycle was 8.8 ± 0.01 days and the total length of spermatogenesis lasted 37.8 ± 0.01 days (4.5 cycles). A high number of germ cell apoptosis was evident during meiosis, resulting in lower Sertoli cell and spermatogenic efficiencies, when compared with laboratory mice strains.

Keywords: Germ cell apoptosis, Leydig cell, Sertoli cell, Sperm production, Spermatogenesis

Introduction

Due to their short reproductive cycle and reduced maintenance cost, mice are widely used as an experimental model in reproductive biology research (Rochester, 2013; Gioiosa *et al.*, 2015). Japanese fancy mouse, mini mouse or pet mouse are common names used to refer to domesticated strains of mice that present with different colour varieties and coat types (Davies, 1912). The Japanese fancy mouse has many coloured spots or patches, irregularly placed on a white background and was established from the original Japanese wild mouse, *Mus musculus molossinus* (Davies, 1912; Minezawa *et al.*, 1979; Koide *et al.*, 1998). Some studies have suggested that this strain is a hybrid between the ancestral colonies of *M. m. musculus* and *M. m. castaneus*, rather than an independent subspecies (Yonekawa *et al.*, 1988).

Although testis anatomy and organization are similar in mammals, each species may present specific morphofunctional features, such as those related to phylogenetic aspects and reproductive strategies (Kerr *et al.*, 2006; Setchell & Breed, 2006; Costa *et al.*, 2010a).

[1]All correspondence to: Luiz Renato de França. Laboratory of Cellular Biology, Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil. Tel: +55 31 96181992 or +55 92 996055655. E-mail: lrfranca@icb.ufmg.br or lrfranca@inpa.gov.br
[2]Laboratory of Cellular Biology, Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.
[3]Federal University of Lavras (UFLA), Animal Science, Lavras, MG, Brazil.
[4]National Institute of Amazonian Research (INPA), Manaus, AM, Brazil.

Comprehensive studies on testis morphology and function have been conducted on very few living mammalian species (Leal & França, 2009; Cordeiro-Júnior *et al.*, 2010; Costa *et al.*, 2010b). Knowledge of male reproduction is crucial for comparative studies (Comizzoli *et al.*, 2000; Pukazhenthi *et al.*, 2006).

Extensive asynapsis of chromosomes in meiosis has been demonstrated for male and female hybrids between two closely related mouse subspecies (leading to pachytene arrest) (Bhattacharyya *et al.*, 2013). Based on the difficulty in generating large cohorts of offspring by natural mating, recently it was reported that the Japanese fancy mouse (hybrid) has poor reproductive performance (Hasegawa *et al.*, 2012). Although many genetic studies involving spotting phenotype on the coat have been performed on fancy mouse (Steingrímsson *et al.*, 2006; Yoshiki & Moriwaki, 2006; Moriwaki *et al.*, 2009; Reissmann & Ludwig, 2013), to our knowledge there are no quantitative data available regarding its testis structure and spermatogenic efficiency. In this context, the main aims of this study were to conduct a detailed testis biometric, histological and stereological analysis as well as to estimate the duration of the spermatogenic cycle, and the Sertoli cell and spermatogenic efficiencies in the Japanese fancy mouse.

Materials and Methods

Animals

Ten sexually mature Japanese fancy mice (JF1/MsJ, ~3.5 months of age) weighing ~15 g were used in this study. The animals were approximately 7–13 cm of length (from the tip of the nose to the tip of the tail) and were obtained from commercial breeders (Sete Lagoas City, Minas Gerais State, Brazil). The typical animal phenotype consisted of a black spotted white coat, and black eyes with a grey-ringed iris (Mouse Phenome Database, The Jackson Laboratory, USA). The animals were kept under controlled temperature (23°C) and lighting conditions (12L:12D photoperiod). Experiments were conducted following approved instructions for the ethical treatment of animals (CEUA/UFMG).

Thymidine injections and tissue processing

To estimate spermatogenesis duration, three animals received i.p. injections of tritiated thymidine (82.0 Ci/mmol, Amersham, Life Science, Buckinghamshire, UK), a tracer for proliferating cells. Injections of 100 μCi ^{3}H-thymidine were performed using a hypodermic needle and the animals that received two thymidine injections were sacrificed at approximately 9 days and 1 h after injections.

Testes were perfused-fixed (through the left ventricle) by gravity with 0.9% saline containing 4% buffered glutaraldehyde, over 30 min (Sprando, 1990). All animals received i.p. injection of heparin (125 IU kg/BW) and pentobarbital (50 mg kg/BW) before surgery. After fixation, testes were weighed, and cut with a razor blade into small fragments. Afterwards, the fragments were immersed, for 12 h, in 4% buffered glutaraldehyde. The tissue samples (~2 mm thickness) were prepared and embedded in plastic (glycol methacrylate) for histological and morphometric analyses.

For the autoradiographic analysis, unstained testis sections were immersed in a specific emulsion (Kodak NTB-2, Eastman Kodak Company Rochester, NY, USA) at 45°C. After drying for 1 h at 25°C, the testis sections were stored at 4°C over the course of 4 weeks. Afterwards, these sections were developed using Kodak D-19 solution (Eastman Kodak Company, Rochester, NY, USA) (Bundy, 1995) and, afterwards, stained with toluidine blue. The subsequent evaluations of the sections were performed to identify the most differentiated germ cells type marked (when five grains were present over the nucleus) (França *et al.*, 1998) after the radioisotope injection.

Testis morphometry

The volume densities of the different testis structures were calculated by light microscopy using a grid (441-intersection) placed in the microscopic ocular. Each animal scored 15 fields (6615 points) during counts at ×400 magnification. The seminiferous tubule diameter, as well as the epithelium height, were measured using a ruler placed in the microscopic ocular. Round tubule cross-sections ($n = 30$) were measured for each animal. The whole length of seminiferous tubules (metres) was achieved using the cylinder formula, considering the seminiferous tubule volume as well as the half of tubular diameter (radius) (Johnson & Neaves, 1981).

Seminiferous epithelium cycle

Seminiferous epithelium cycle (SEC) stages were characterized using acrosome and spermatid nuclear morphology. Stage frequencies were calculated analysing 150 seminiferous tubule cross-sections per animal. The spermatogenic cycle duration was estimated taking into consideration the most differentiated germ cell type marked at different periods (times) after radioisotope injection. The duration of spermatogenesis was estimated taking into account that 4.5 cycles are necessary for the completion of the entire process (Amann, 1983).

https://doi.org/10.1017/S0967199417000326 Published online by Cambridge University Press

Cell counts and cell numbers

Germ and Sertoli cells

All Sertoli cell nucleoli and germ cell nuclei were counted per animal in 10 round seminiferous tubule cross-sections at stage VII of the cycle. These counts were normalized considering the section thickness (4 μm) and nucleus/nucleolus diameter (Abercrombie, 1946; Amann, 1962). The Sertoli cell nucleolus diameter (10 nucleoli per animal) was measured because it makes the identification of this cell easier as the nuclear shape is irregular. In addition, the germ cells nuclei (n = 10) were measured per animal and the cell ratios were found from the normalized counts.

Considering that there are no significant apoptosis during spermiogenesis (Russell & Clermont, 1977), the round spermatids number obtained in this species was considered to be the final population of spermatozoa. Sertoli cell number was calculated for each testis and per testis gram from the normalized counts of Sertoli cell nucleoli and the seminiferous tubules length (Hochereau-de-Reviers & Lincoln, 1978). The daily sperm production for each testis and per testis gram was calculated based on Sertoli cell number, efficiency and seminiferous stage frequencies using the formula described by França (1992).

Leydig cells

The proportion between nucleus and cytoplasm was calculated to estimate the individual Leydig cell volume. Its volume was calculated from the mean nuclear diameter as the nucleus is spherical. In this regard, 30 Leydig cell nuclei were measured per animal and the nuclear volume was achieved using the volume formula of a sphere, in which radius = nuclear diameter/2. The nuclear and cytoplasmic percentages were obtained placing a grid (1000 points) over the Leydig cells. The individual cell size and the entire volume occupied by these cells in the testis parenchyma allowed the achievement of the Leydig cell number per testis and per gram of testis.

Table 1 Stereological biometric and testis data in fancy mice [mean ± standard error of the mean (SEM)]

Parameters	Fancy mice
Body weight (g)	14.6 ± 0.6
Testis weight (mg)	68.2 ± 5.5
Gonadosomatic index (%)	0.94 ± 0.01
Testis parenchyma volume density (%)	
Tubular compartment	94.2 ± 0.4
Tunica propria	5.3 ± 0.1
Seminiferous epithelium	76.3 ± 0.2
Lumen	12.6 ± 0.2
Intertubular compartment	5.8 ± 0.4
Leydig cell	4.5 ± 0.4
Blood vessels	0.1 ± 0.1
Connective tissue/lymphatic space	1.2 ± 0.4
Tubular diameter (μm)	205 ± 3
Seminiferous epithelium height (μm)	64.5 ± 1.6
Tubular length per gram of testis (m)	28.5 ± 0.8
Total tubular length per testis (m)	1.9 ± 0.1

Table 2 Stages of the seminiferous epithelium cycle and relative stage frequencies in Japanese fancy mice (mean ± SEM)

Stages of the seminiferous epithelium cycle	Relative stage frequencies (%)
I	17.2 ± 0.3%
II-III	3.9 ± 0.1%
IV	3.5 ± 0.1%
V	3.7 ± 0.1%
VI	5.9 ± 0.2%
VII	23.1 ± 0.5%
VIII	7.1 ± 0.2%
IX	9.4 ± 0.3%
X	7.6 ± 0.2%
XI	6.9 ± 0.1%
XII	11.7 ± 0.3%
Pre-meiotic (Stages IX to XI)	23.9 ± 0.3%
Meiotic (Stage XII)	11.7 ± 0.3%
Post-meiotic (Stages I to VIII)	64.4 ± 0.5%

Results

Biometric data and testis volume density

Mean body and testis weights were ~15 g and 68 mg respectively, giving almost 1% of gonadosomatic index (testis mass in relation to body weight) (Table 1). As shown in Table 1, the tunica albuginea percentage (data obtained after the removal of testis parenchyma) was approximately 4%, while the volume density of the tubular and intertubular compartments were ~94% and 6%, respectively. In these compartments, the seminiferous epithelium occupied ~76% while the Leydig cells volume occupancy was 4.5% (Fig. 1). The tubular diameter and height of seminiferous epithelium were 205 and 65 μm respectively (Table 1). The length of the seminiferous tubule per gram of testis was approximately 29 m, whereas about 2 m of tubules were found per testis.

Stages of seminiferous epithelium cycle and their relative frequencies

Twelve stages of SEC were classified based on acrosome morphology in spermatids (Fig. 2). The germ cell composition in the 12 stages and their relative frequencies are shown in Fig. 3 and Table 2. The

https://doi.org/10.1017/S0967199417000326 Published online by Cambridge University Press

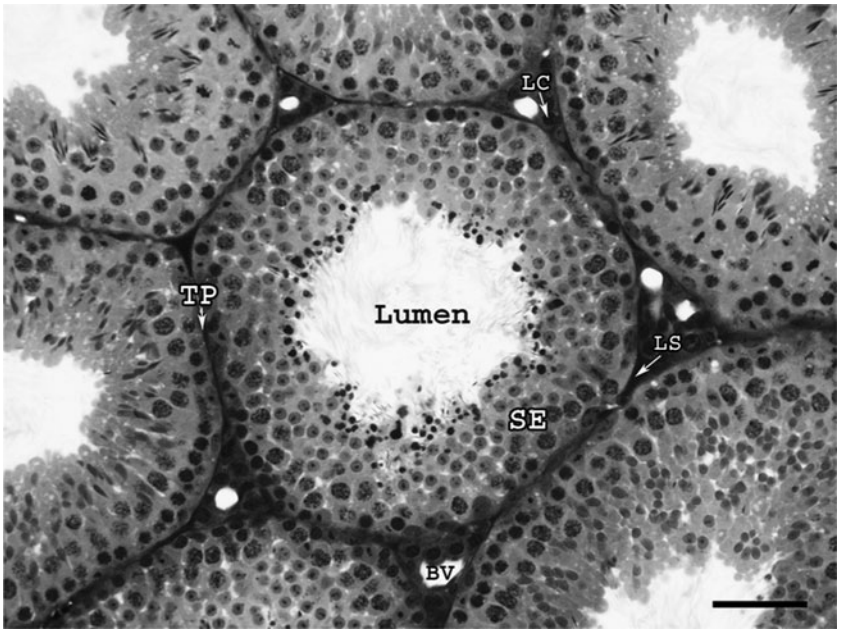

Figure 1 Cross-section showing tubular and interstitial compartments of the Japanese fancy mouse testis. The tubular compartment is comprised by tunica propria (TP), seminiferous epithelium (SE) and lumen. In the intertubular compartment, the Leydig cells (LC) are closely associated with the blood vessels (BV) and surrounded by a lymph-filled space (LS). Figure magnification = ×200; scale bar = 50 μm.

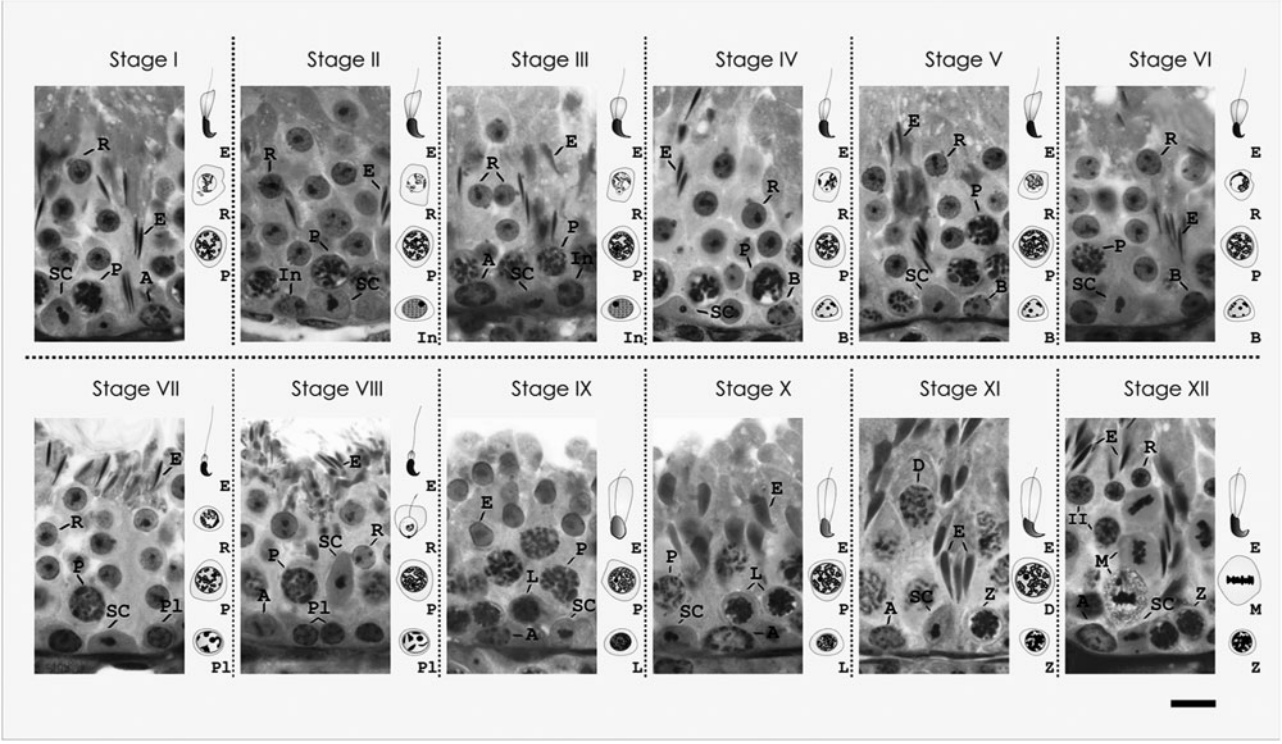

Figure 2 Stages I to XII of the seminiferous epithelium cycle, based on the development of the acrosome, in spermatids and overall germ cell association are shown in Japanese fancy mouse. The individual germ cell nuclei shown in the right column represent the germ cells found in each particular stage. Type A spermatogonia (A); intermediate spermatogonia (In); Type B spermatogonia (B); pre-leptotene (Pl); leptotene (L); zygotene (Z); pachytene (P); diplotene (D) spermatocytes; meiotic figure (M); secondary spermatocyte (II); round spermatids (R); elongating/elongated spermatids (E); and Sertoli cell (SC). Figure magnification = ×1000, scale bar = 10 μm.

https://doi.org/10.1017/S0967199417000326 Published online by Cambridge University Press

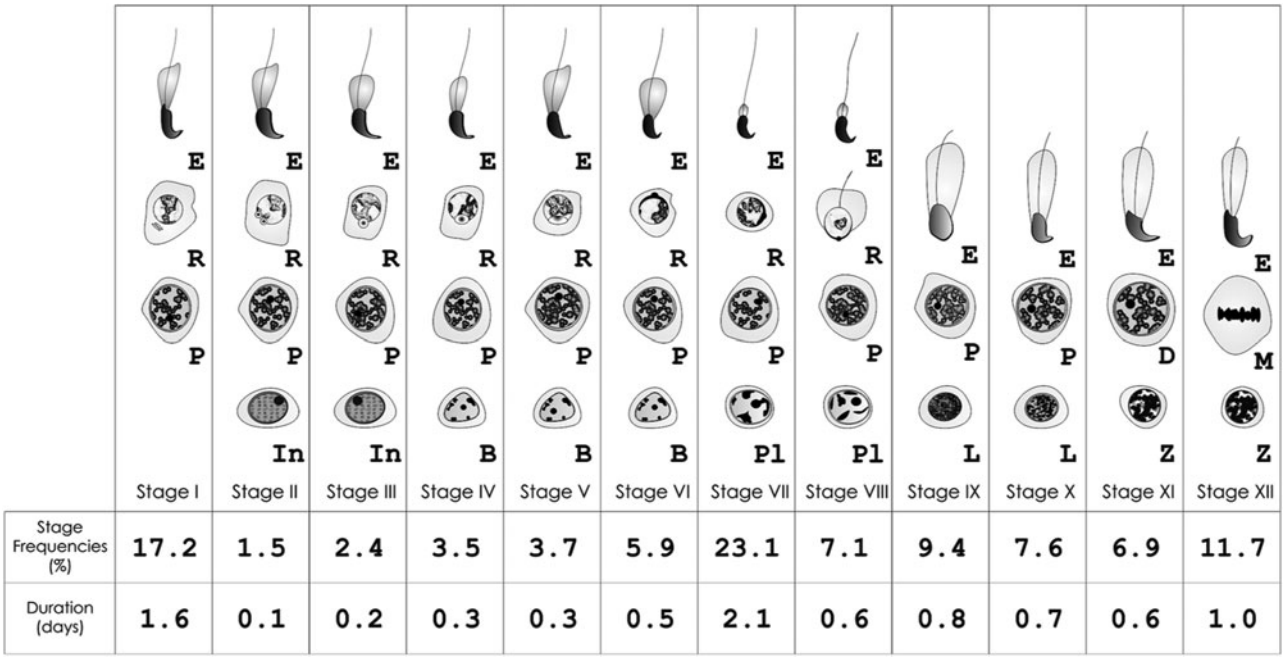

Figure 3 Diagram showing the germ cell composition for each of the 12 stages (roman numerals at the bottom) of the cycle, characterized based on the development of the acrosome in the spermatids nuclei. Letters within each column indicate the different germ cell types present at each stage of the cycle. Intermediate (In) and Type B (B) spermatogonia; preleptotene (Pl), leptotene (L), zygotene (Z), pachytene (P) and diplotene spermatocytes (D); meiotic figures (M); round (R) and elongating/elongated spermatids (E). The stage frequencies and duration (in days) of each stage are also shown at the bottom in this figure.

Table 3 The length (days) of seminiferous epithelium cycle (mean ± SEM)

Mice	Time after injection	Most advanced germ cell type labelled	Stage of the cycle	Number of cycles traversed	Cycle length based on labelling in preleptotene
1	1 h	Pl[a]	VIII	–	–
	8.844 days[c]	P[b]	VIII	1	8.844
2	1 h	Pl[a]	VIII	–	–
	8.842 days[c]	P[b]	VIII	1	8.842
3	1 h	Pl[a]	VIII	–	–
	8.845 days[c]	P[b]	VIII	1	8.845

Mean duration of the cycle = 8.84 ± 0.01 days.
[a]Pl/L, Preleptotene primary spermatocytes.
[b]P, pachytene spermatocytes.
[c]Total time after thymidine injection minus 1 h.

frequencies of stages II and III were presented together because of their very low occurrence and stage VII (~23%) showed the highest frequency. The frequencies of pre-meiotic (stages IX–XI), meiotic (stage XII) and post-meiotic (I–VIII) phases were approximately 24%, 12% and 64% respectively.

Seminiferous epithelium cycle duration

The most differentiated germ cell types (3H-thymidine positive) observed at the different time periods evaluated post radioisotope administrations, are shown in Fig. 4 and Table 3. Approximately 1 h after injection, the labelled cells were preleptotene spermatocytes that were located in the basal compartment at stage VIII (Fig. 4). Nine days after injection, labelled pachytene spermatocytes were found in the same stage (Fig. 4). Considering the most advanced labelled germ cell, the SEC duration was calculated as 8.8 ± 0.01 days. In our study, the interval between the germ cells labelling (preleptotene to pachytene spermatocytes) corresponded to approximately one cycle of the seminiferous epithelium (Fig. 4). Taking into consideration that approximately 4.5 cycles are

https://doi.org/10.1017/S0967199417000326 Published online by Cambridge University Press

Table 4 Cell counts, cell ratios and sperm production in Japanese fancy mouse (mean ± SEM)

Parameters	
Round spermatids: pachytene spermatocyte	2.07 ± 0.03: 1
Round spermatids: Sertoli cell nucleoli	7.9 ± 0.3: 1
Sertoli cell number per gram of testis (million)	42.4 ± 1.2
Sertoli cell number per testis (million)	2.8 ± 0.2
Daily sperm production per gram of testis (million)	38.7 ± 1.3
Daily sperm production per testis (million)	2.6 ± 0.2

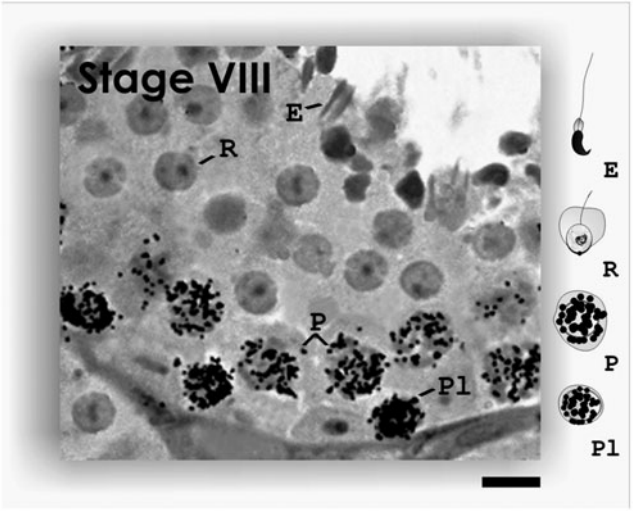

Figure 4 Most advanced labelled germ cells found at the two different time intervals after thymidine injections in Japanese fancy mouse. As can be observed the labelled cells were preleptotene (Pl) and pachytene (P) spermatocytes found at the stage VIII respectively at 1 h and 8.8 days. R = round spermatids; E = elongated spermatids. Figure magnification = ×1000, scale bar = 10 μm.

required for the entire spermatogenesis, the total length of this process was estimated to be 39.8 ± 0.01 days.

Testis stereology

The data related to the testis stereology are displayed in Tables 4 and 5. The number of round spermatids that originated from each pachytene primary spermatocyte (meiotic index), was approximately 2.1. This result demonstrated that almost 50% of germ cell loss occurred in the course of the two meiotic divisions (Fig. 5). Almost eight round spermatids are supported by each Sertoli cell (Sertoli cell efficiency). The Sertoli cell number per testis gram was 42×10^6, whereas this

Table 5 Leydig cell morphometry in Japanese fancy mice (mean ± SEM)

Parameters	
Nuclear diameter (μm)	6.9 ± 0.1
Leydig cell volume (μm³)	604 ± 14
Nucleus volume (μm³)	175 ± 4
Cytoplasm volume (μm³)	429 ± 11
Leydig cell number per testis (million)	4.7 ± 0.4
Leydig cell number per gram of testis (million)	74.6 ± 5.9

figure per testis was approximately 3×10^6. The daily sperm production per testis gram and per testis were about 39×10^6 and 3×10^6 respectively. The Leydig cell nuclear volume was 175 μm^3 while its individual size was 604 μm^3. The data obtained for Leydig size and volume occupancy allowed us to estimate that the number of this steroidogenic cell per gram of testis (~75 millions) and per testis (~5 millions).

Discussion

This study is the first to perform a detailed stereological/morphometric evaluation of the testis and to estimate testis key parameters such as the duration of spermatogenesis, Sertoli cell and spermatogenic efficiencies in the Japanese fancy mice. We demonstrated a high index of apoptotic spermatocytes during the meiotic divisions and, as a consequence of this germ cell loss, the values found for Sertoli cell and spermatogenic efficiencies were lower when compared with other laboratory mice strains investigated (Clermont & Trott, 1969; Avelar *et al.*, 2004).

Table 6 is a summary of key parameters comparing the data herein obtained with those related to laboratory mice strains (Clermont & Trott, 1969; Avelar *et al.*, 2004; Hess & França, 2007). However, the spermatogenic efficiency in the Japanese fancy mice is still relatively high in comparison with other evaluated mammalian species (França & Russell, 1998; Johnson *et al.*, 2000; França *et al.*, 2005; Hess & França, 2007).

Sertoli cell number (Sharpe *et al.*, 2003; França *et al.*, 2005; Holsberger & Cooke, 2005) determines the magnitude of sperm production per testis (spermatogenic efficiency) (Johnson *et al.*, 2000; Hess & França, 2007). Therefore, this cell number was used to quantify and analyse daily sperm production, as Sertoli cell efficiency is relatively constant for each species (França & Russell, 1998; Johnson *et al.*, 2000; França & Hess, 2005; França *et al.*, 2005; Gerber *et al.*, 2016). Although Sertoli cell number per gram of testis found in Japanese fancy mouse is very similar to that obtained for laboratory mice, probably due to high incidence of apoptosis in the second phase of the

https://doi.org/10.1017/S0967199417000326 Published online by Cambridge University Press

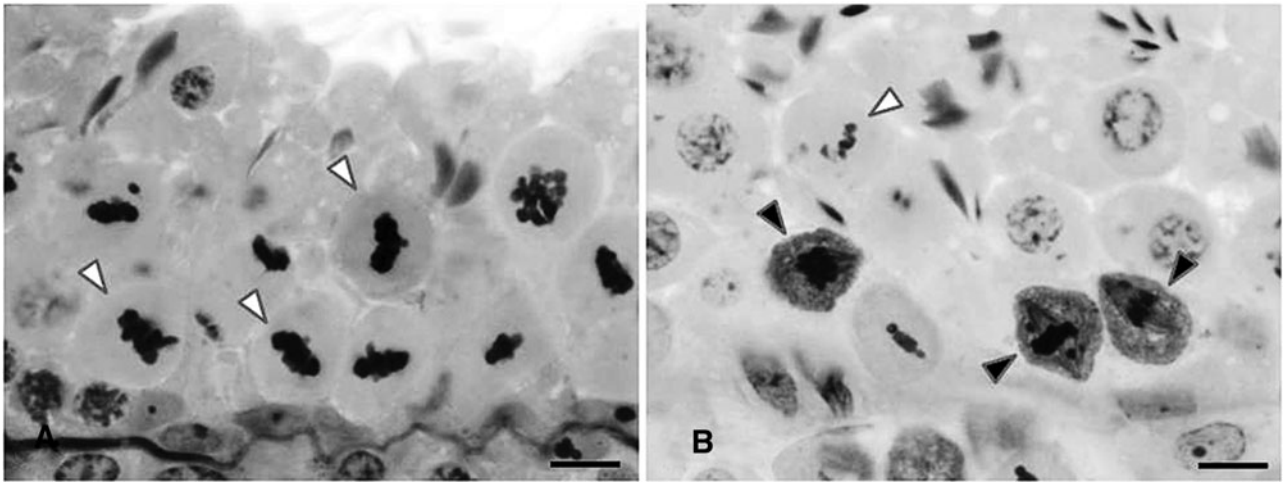

Figure 5 Germ cell apoptosis during meiotic divisions in the Japanese fancy mouse. Seminiferous tubule cross-sections displaying morphologically normal meiotic divisions (A; white arrowheads) and commonly observed apoptotic meiotic figures (B; black arrowheadheads). Figure magnification = ×1000, scale bar = 10 μm.

Table 6 Comparative parameters related to the testis stereology and spermatogenic events

Parameters	Japanese fancy mice	Laboratory mice[a]
Body weight (g)	15	26–39
Testis weight (mg)	68	95–113
Gonadosomatic index (%)	0.94	0.76–0.55
Seminiferous tubule (%)	94	91–93
Leydig cell (%)	4.5	3.7–5.3
Leydig cell size (μm^3)	604	1021–1450
Leydig cell number per gram of testis ($x10^6$)	75	29–49
Pre-meiotic phase[b](%)	24	22
Meiotic phase[c](%)	12	9
Post-meiotic phase[d] (%)	64	69
Meiotic index[e]	2.1 (48%)	2.3–2.8 (43–30%)
Spermatogenic cycle length (days)	8.8	8.6–8.9
Total duration of spermatogenesis (days)	39.8	38.7–40
Sertoli cells per gram of testis ($x10^6$)	42	39–41
Round spermatids per Sertoli cell	8	10.5–11.5
Daily sperm production per gram of testis ($x10^6$)	39	45–48

[a]Clermont & Trott, 1969; Avelar *et al.*, 2004; Hess & França, 2007.
[b]After spermiation and prior to meiosis.
[c]Meiosis I through meiosis II.
[d]After completion of meiosis until spermiation.
[e]Measured as the number of round spermatids produced per pachytene primary spermatocyte (presumptive germ cell loss in parenthesis).

spermatogenesis the values obtained for Sertoli cell efficiency in this hybrid are lower. The data obtained here corroborate with the data of Bhattacharyya *et al.* (2013) in which meiotic asynapsis of heterospecific homologous chromosomes was found for hybrids of mice (*Mus m. musculus* × *Mus m. domesticus*) leading to pachytene arrest and lower reproductive rates. This situation probably occurs because meiosis is carefully monitored through checkpoints, avoiding therefore the creation of defective gametes with an aberrant number of chromosomes (Subramanian & Hochwagen, 2014).

Regarding the stage frequencies, our study corroborates with several studies suggesting that phylogenetically close related species present a similar stage frequencies distribution when these frequencies are grouped in pre-meiotic and post-meiotic phases (Hess & França, 2007; Costa *et al.*, 2010a, b). For instance, as shown in Table 6 the frequencies of these phases are very close when different mice strains are compared. Although the length of spermatogenesis is controlled by the germ cell genome (França *et al.*, 1998) and not phylogenetically determined (Clermont, 1972; Amann

https://doi.org/10.1017/S0967199417000326 Published online by Cambridge University Press

& Schanbacher, 1983), the values observed for the SEC duration are very similar among fancy and laboratory mice strains (Table 6).

Regarding testis interstitium, the fancy mouse followed the pattern of organization already described for rodents, i.e. presented extensive peritubular lymphatic sinusoids and groups of Leydig cells clustered with blood vessels (Kerr *et al.*, 2006). Although the Leydig cell morphology and volumetric density are very similar between fancy and laboratory mouse, the individual Leydig cell volume in fancy mouse represents approximately half of the cell volume observed for laboratory mice. This characteristic leads therefore to approximately two-fold higher number of these steroidogenic cells per gram of testis in the fancy mouse (Clermont & Trott, 1969; Avelar *et al.*, 2004). To our knowledge, an explanation about what determines the Leydig cell volume density and its size is not available in the literature.

In conclusion, most of the testis parameters investigated in this study for Japanese fancy mice were similar to those obtained for laboratory mice. Although there is a high incidence of germ cell loss during the meiotic divisions, the combination of high tubular compartment volume, fast duration of spermatogenesis and high number of Sertoli cells allows high sperm production in the Japanese fancy mouse, though not as high as the laboratory mice.

Author contributions

GMJC, MCL and LRF performed the experiments, analysed the data and wrote the paper.

Conflict of interest statement

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

Technical help from Mara L. Santos is highly appreciated.

Financial support

This work was supported by the Brazilian National Council for Research (CNPq); Minas Gerais State Foundation (FAPEMIG); and Coordination for the Improvement of Higher Level Personnel (CAPES) and PRPq.

References

Abercrombie, M. (1946). Estimation of nuclear populations from microtome sections. *Anat. Record.* **94**, 238–48.

Amann, R.P. (1962). Reproductive capacity of dairy bulls. IV. Spermatogenesis and testicular germ cell degeneration. *Am. J. Anat.* **110**, 69–78.

Amann, R.P. & Schanbacher, B.D. (1983). Physiology of male reproduction. *J. Anim. Sci.* **2**, 380–403.

Amann, R.P., 1983. Endocrine changes associated with onset of spermatogenesis in Holstein bulls. *J. Dairy Sci.* **66**, 2606–22.

Avelar, G.F., Leal, M.C. & França, L.R. (2004). Sertoli and Leydig cells number per testis and daily sperm production in different mice strains. In *13th European Workshop on Molecular & Cellular Endocrinology of Testis* (eds F.O. Levy, K. Taskén & V. Hansson), p. H1. Dunblane, Scotland.

Bhattacharyya, T., Gregorova, S., Mihola, O., Anger, M., Sebestova, J., Denny, P., Simecek, P. & Forejt, J. (2013). Mechanistic basis of infertility of mouse intersubspecific hybrids. *Proc. Natl. Acad. Sci. USA* **110**, E468–77.

Bundy, D.C. (1995). Photographic emulsions and processing. In *Autoradiography and Correlative Imaging* (eds W.E. Stumpf & H.F. Solomon), pp: 49–57. San Diego: Academic Press.

Clermont, Y., Trott, M. (1969). Duration of the cycle of the seminiferous epithelium in the mouse and hamster determined by means of 3H-thymidine and radioautography. *Fertil. Steril.* **20**, 805–17.

Clermont, Y. (1972). Kinetics of spermatogenesis in mammals seminiferous epithelium cycle and spermatogonial renewal. *Physiol. Rev.* **52**, 198–236.

Comizzoli, P., Mermillod, P. & Mauget, R. (2000). Reproductive biotechnologies for endangered mammalian species. *Reprod. Nutr. Dev.* **40**, 493–504.

Cordeiro-Júnior, D.A., Costa, G.M., Talamoni, S.A. & França, L.R. (2010) Spermatogenic efficiency in the spiny rat, *Trinomys moojeni* (Rodentia: Echimyidae). *Anim. Reprod. Sci.* **119**, 97–105.

Costa, G., Leal, M., Silva, J., Ferreira, A.C., Guimarães, D.A. & França, L.R. (2010a). Spermatogenic cycle length and sperm production in a feral pig species (collared peccary, *Tayassu tajacu*). *J. Androl.* **31**, 221–30.

Costa, G., Leal, M., Ferreira, A.C., Guimarães, D.A. & França, L.R. (2010b). Duration of spermatogenesis and spermatogenic efficiency in two large neotropical rodent species: the agouti (*Dasyprocta leporina*) and paca (*Agouti paca*). *J. Androl.* **31**, 489–99.

Davies, C.J. (1912). *Fancy Mice, Their Varieties and Management as Pets or for Show*. London: Cornell University Library.

França, L.R. & Hess, R.A. (2005). Structure of the Sertoli cell. In *Sertoli Cell Biology* (eds M. Skinner & M. Griswold), pp: 19–40. San Diego: Elsevier Academic Press.

França, L.R. & Russell, L.D. (1998). The testis of domestic animals, In *Male Reproduction: A Multidisciplinary Overview* (eds F. Martýnez & J. Regadera), pp. 197–219. Madrid: Churchill Livingston.

França, L.R., Ogawa, T., Avarbock, M.R., Brinster, R.L. & Russell, L.D. (1998). Germ cell genotype controls cell cycle during spermatogenesis in the rat. *Biol. Reprod.* **59**, 1371–7.

https://doi.org/10.1017/S0967199417000326 Published online by Cambridge University Press

França, L.R., Avelar, G.F. & Almeida, F.F. (2005). Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. *Theriogenology* **63**, 300–18.

França, L.R. (1992). Daily sperm production in Piau boars estimated from Sertoli cell population and Sertoli cell index, In *Proceedings of the 12th International Congress on Animal Reproduction and Artificial Insemination* (ed. S. J. Dieleman), pp. 1716–8. The Netherlands: Elsevier Science.

Gerber, J., Heinrich, J. & Brehm, R. (2016). Blood–testis barrier and Sertoli cell function: lessons from SCC×43KO mice. *Reproduction* **151**, R15–27.

Gioiosa, L., Palanza, P., Parmigiani, S. & vom Saal, F.S. (2015). Effects of developmental exposure to low doses of bisphenol A on behavior and physiology in mice (*Mus musculus*). *Dose Response* **13**, 1–8.

Hasegawa, A., Mochida, K., Matoba, S., Yonezawa, K., Ohta, A., Watanabe, G., Taya, K. & Ogura, A. (2012). Efficient production of offspring from Japanese wild-derived strains of mice (*Mus musculus molossinus*) by improved assisted reproductive technologies. *Biol. Reprod.* **86**, 1–7.

Hess, R.A. & França, L.R. (2007). Spermatogenesis. Cycle of the seminiferous epithelium, In *Molecular Mechanisms in Spermatogenesis* (ed. C.Y. Cheng), pp. 1–15. Austin, USA: Landes Bioscience.

Hochereau-de-Reviers, M.T. & Lincoln, G.A. (1978). Seasonal variation in the histology of the testis of the red deer, *Cervus elaphus*. *J. Reprod. Fertil.* **54**, 209–13.

Holsberger, D.R. & Cooke, P.S. (2005). Understanding the role of thyroid hormone in Sertoli cell development: a mechanistic hypothesis. *Cell Tissue Res*. **322**, 133–40.

Johnson, L. & Neaves, W.B. (1981). Age-related changes in the Leydig cell population, seminiferous tubules and sperm production in stallions. *Biol. Reprod.* **24**, 703–12.

Johnson, L., Varner, D.D., Robert, M.E., Smith, T.L., Keillor, G.E. & Scrutchfield, W.L. (2000). Efficiency of spermatogenesis: a comparative approach. *Anim. Reprod. Sci.* **61**, 471–80.

Kerr, J.B., Loveland, K.L., O'Bryan, M.K. & Kretser, D.M. (2006). Cytology of the testis and intrinsic control mechanisms, In: *Physiology of Reproduction* (ed. J.D. Neill), pp. 827–947. Birmingham, UK: Elsevier, Birmingham.

Koide, T., Moriwaki, K., Uchida, K., Mita, A., Sagai, T., Yonekawa, H., Katoh, H., Miyashita, N., Tsuchiya, K., Nielsen, T.J. & Shiroishi, T. (1998). A new inbred strain JF1 established from Japanese fancy mouse carrying the classic piebald allele. *Mamm. Genome* **9**, 15–9.

Leal, M.C. & França, L.R. (2009). Slow increase of Sertoli cell efficiency and daily sperm production causes delayed establishment of full sexual maturity in the rodent *Chinchilla lanigera*. *Theriogenology* **71**, 509–18.

Minezawa, M., Moriwaki, K. & Kondo, K. (1979). Geographical distribution of HbbP alleles in the Japanese wild mouse, *Mus musculus molossinus*. *Jpn. J. Genet.* **54**, 165–73.

Moriwaki, K., Miyashita, N., Mita, A., Gotoh, H., Tsuchiya, K., Kato, H., Mekada, K., Noro, C., Oota, S., Yoshiki, A., Obata, Y., Yonekawa, H. & Shiroishi, Y. (2009). Unique inbred strain MSM/Ms established from the Japanese wild mouse. *Exp. Anim.* **58**, 123–34.

Pukazhenthi, B., Comizzoli, P., Travis, A.J. & Wildt, D.E. (2006). Applications of emerging technologies to the study and conservation of threatened and endangered species. *Reprod. Fertil. Dev.* **18**, 77–90.

Reissmann, M. & Ludwig, A. (2013). Pleiotropic effects of coat colour-associated mutations in humans, mice and other mammals. *Semin. Cell Dev. Biol.* **24**, 576–86.

Rochester, J.R. (2013). Bisphenol A and human health: a review of the literature. *Reprod. Toxicol.* **42**, 132–55.

Russell, L.D. & Clermont, Y. (1977). Degeneration of germ cells in normal, hypophysectomized and hormone treated hypophysectomized rats. *Anat. Rec.* **187**, 347–66.

Setchell, B.P. & Breed, W.G. (2006). Anatomy, vasculature and innervation of the male reproductive tract. In *Physiology of Reproduction* (ed. J.D. Neill), pp. 771–825. Birmingham, UK: Elsevier.

Sharpe, R.M., Fraser, H.M., Brougham, M.F., McKinnell, C., Morris, K.D., Kelnar, C.J., Wallace, W.H. & Walker, M. (2003). Role of the neonatal period of pituitary-testicular activity in germ cell proliferation and differentiation in the primate testis. *Hum. Reprod.* **18**, 2110–7.

Sprando, R.L. (1990). Perfusion of the rat testis through the heart using heparin. In *Histological and Histopathological Evaluation of the Testis* (eds L.D. Russell, R.A. Ettlin, A.P. Sinha-Hikim. & E.D. Clegg), pp. 277–80. Clearwater: Cache River Press.

Steingrímsson, E., Copeland, N.G. & Jenkins, N.A. (2006). Mouse coat colour mutations: from fancy mice to functional genomics. *Dev. Dyn.* **235**, 2401–2411.

Subramanian, V.V. & Hochwagen, A. (2014). The meiotic checkpoint network: step-by-step through meiotic prophase. *Cold Spring Harb. Perspect. Biol.* **6**, a016675.

Yonekawa, H., Moriwaki, K., Gotoh, O., Watanabe, J., Hayashi, J.I., Miyashita, N., Petras, M.L. & Tagashira, Y. (1988). Hybrid origin of Japanese mice Mus musculus molossinus: evidence from restriction analysis of mitochondrial DNA. *Mol. Biol. Evol.* **5**, 63–78.

Yoshiki, A. & Moriwaki, K. (2006). Mouse phenome research: implications of genetic background. *ILAR J.* **47**, 94–102.