Original article

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Morphological development of the testicles and spermatogenesis in guinea pigs (*Cavia porcellus* Linnaeus, 1758)

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Abstract

Introduction: Understanding the dynamics of spermatogenesis is crucial to clinical andrology and to understanding the processes which define the ability to produce sperm. However, the entire process cannot be modeled *in vitro* and guinea pig may be an alternative as animal model for studying human reproduction. **Objective:** In order to establish morphological patterns of the testicular development and spermatogenesis in guinea pigs, we examined testis to assess changes in the testis architecture, transition time from spermatocytes to elongated spermatids and stablishment of puberty. **Materials and methods:** We used macroscopic analysis, microstructural analysis and absolute measures of seminiferous tubules by light microscopy in fifty-five guinea pigs from one to eleven weeks of age. **Results:** Differences in relation to mass, length and width of the testes appeared at week 3 and were intensified in week 6 with the puberty. At week 2 the first spermatocytes at preleptotene/leptotene appeared, indicating the onset of meiosis. At week 6 the seminiferous tubules formed lumen, differentiated Sertoli cells and all kinds of germ cells; spermatozoa released into the lumen and the largest expansion of tubular diameter and height of the seminiferous epithelium occurred. Transition time from spermatocytes to elongated spermatids was estimated in 21 days. **Conclusion:** We conclude that the testicular development and puberty are faster than in other mammal and rodent species, demonstrating the suitability of the guinea pig testes as a model for studies of the testicular development and spermatogenesis.

Keywords: sexual development, germ cells, spermatocyte, seminiferous epithelium.

1 Introduction

Given the complexity of male fertility, the entire process cannot be modeled *in vitro* and rodents have collaborated to the growth of science, because some species show up as excellent experimental models (SIMMONS, 2008; ISLAM and WILSON, 2012; JOHNSON, 2012). Among rodents the guinea pig may be an alternative as animal model for studying human reproduction, by presenting a long gestational period, spontaneous ovulation, and active corpus luteum (SUZUKI, KOURA, NOGUCHI et al., 2003). Some information having been found regarding the accessory glands (GRADELA, NUNES, MARTINS et al., 2013a; GRADELA, NUNES, MATOS et al., 2013b), characterization of mammalian early meiotic gene expression (RODRIGUEZ-CASURIAGA, GEISINGER, SANTINAQUE et al., 2011), biochemical and molecular analysis of early meiotic prophase stages (RODRIGUEZ and WETTSTEIN 2004) and morphological and functional analysis of spermatogenesis (NUNES, GOUVEIA, MATOS et al., 2013).

Although the organization of spermatogenesis is similar among the various mammalian species, daily sperm production in each species may be influenced by specific characteristics related to the volume proportion occupied by the components of testicular parenchyma, the number of spermatogonial generations, the sequence of proliferation and morphology of cells in the seminiferous epithelium, the chronology of vacuolation and lumen formation in testicular cords, and the overall spermatogenesis yield (FRANÇA and RUSSELL, 1998). Primates and most rodents differ, because in the former there is a clear period of separation between postnatal and pubertal periods, with the spermatogenesis starting many months or years after birth. This does not occur in mice and others rodents, in which the spermatogenesis begins within a few days after birth (MONTOTO, ARREGUI, SÁNCHEZ et al., 2012) and the full fertility can be seen at 6–7 weeks of age (BORG, WOLSKI, GIBBS et al., 2010). Thus, the morphology and function of the testis that is observed in adult males develops

during a protracted period of time before and during puberty (KERR, LOVELAND, O'BRYAN et al., 2006).

Sperm production and testicular size are highly correlated, hence, testicular size constitutes a quantitative indicator of sperm production (FRANÇA and RUSSELL, 1998; JOHNSON, VARNER, ROBERTS et al., 2000) and it is a useful tool for evaluation. Moreover, establishing the relation between testicular and body size, known as gonadosomatic index (GSI) it is possible to obtain information on the reproductive physiology and mating system of a species (CALDEIRA, PAULA, MATTA et al., 2010). Furthermore, longitudinal studies are considered an excellent approach to investigate postnatal testis development and particularly the aspects related to the establishment of puberty and sexual maturity, crucial parameters in determining the magnitude of sperm production (LEAL and FRANÇA, 2008). A positive correlation has been observed, in many species, among all quantitative data related to seminiferous tubules and spermatogenic activity (FRANÇA and RUSSELL, 1998; SILVA JUNIOR, VIEIRA, PAULA et al., 2006; CALDEIRA, PAULA, MATTA et al., 2010). Furthermore, the study of the first wave of spermatogenesis can represent an outstanding model for the characterization of gene expression, hormone regulation and for the local factor control during germ cell proliferation, meiosis, and postmeiotic differentiation under different levels of sperm competition (MONTOTO, ARREGUI, SÁNCHEZ et al., 2012).

The aim of the present study was investigate postnatal phases of the development and testis differentiation in guinea to assess changes in testis architecture, transition time from spermatocytes to elongated spermatids and the establishment of puberty. For that we evaluated the development of the body and testes, gonadosomatic index, tubular diameter and height of the seminiferous epithelium and the cell populations present in the seminiferous epithelium.

2 Materials and Methods

2.1 Animals and sample collection

Fifty-five guinea pigs (*Cavia porcellus*) born and bred in the vivarium of UNIVASF in Petrolina, Pernambuco, Brazil (Latitude 09°23'55"; longitude 40°30'03"; altitude 376 m) were divided according to age, in weeks, into 11 groups, from week 1 to week 11, with 5 animals in each group. After weaning, at the 4th week of life, they received water and commercial feed *ad libitum* and were kept under standard laboratory conditions in environmentally controlled rooms at 20-24 °C, until the evaluation time. All procedures utilized followed approved guidelines for the ethical treatment of animals, according to the rules of the Ethics Committee for Experimental Human and Animal Research (CEEHA) of the Federal University of São Francisco Valley (UNIVASF), under the Protocol 0001/160412 that follows standard international rules.

After reaching the scheduled age, guinea pigs' body mass (BM) was evaluated on an analytical digital weighing scale. After anesthesia with xylazine hydrochloride (Calmium, União Química, São Paulo, Brazil) and ketamine hydrochloride (Ketamina Agener, Agener União, São Paulo, Brazil) at a 1:5 dilution (0.1 mL/100 g LW) [IM] associated to tramadol hydrochloride (2 mg/1000 g) (Tramal, Teuto e Medley, São Paulo, Brazil) [IM], and euthanasia by exsanguination, we evaluated testicular mass (TM, g), on an analytical digital weighing scale KERN 430-21 max 50 g (d = 0.001 g), and length (TL, cm), width (TW, cm), and thickness (TT, cm) of both testes with a millimeter caliper. Gonadosomatic index (GSI) was calculated for each age group, to quantify the investment in testis tissue in relation to body mass, by the formula: GSI = [mass of both testes / body mass] x 100.

The right testis in three animals from each group (n = 33) was sectioned into three regions (capitate end, middle portion and caudate end), and, subsequently, these fragments were fixed in 10% buffered formalin solution during 18 hours, transferred to a 70% ethanol solution and maintained in the latter until being processed. Testes were embedded in paraffin, sectioned transversally (7 µm thickness, microtome EasyPath, Sao Paulo, Brazil) and, after being deparaffinized, rehydrated and then stained with Mayer's haematoxylin. After gradual dehydration in 70% and 95% ethanol, the sections were further stained in eosin buffer before being mounted. The slides, once stained and assembled, were analyzed by means of a binocular microscope Olympus BX 50 equipped with digital camera (Digital Sight, DS-5M, Nikon, Tokyo, Japan).

2.2 Histological evaluation of testicular development

In 20 transversal sections of testicular cords and/or seminiferous tubules (enhanced 50 x, 100 x, 200 x, and 400 x), we evaluated the lumen formation process in seminiferous tubules; presence or absence of gonocytes, spermatogonia A, primary spermatocytes, and spermatids round or elongated. These data enabled the classification of animals according to the testicular development phases: impuberal, early prepuberal, late prepuberal, puberal, postpuberal 1, and postpuberal 2 (COUROT, HOCHEREAUA-DE REVIERS and ORTAVANT, 1970).

2.3 Absolute measures of seminiferous tubules

We obtained the following measures from the cords and/or seminiferous tubule cross-sections: the diameter of cords and/or seminiferous tubules (µm) and the seminiferous epithelium height (µm). The average diameter of testicular cords and/or seminiferous tubules (TD) was obtained from 10 transversal sections of seminiferous tubules for each animal with complete spermatogenic activity (puberal, postpuberal 1 and postpuberal 2) and from 20 sections for those which did not have all cells of the spermatogenic lineage (impuberal, early prepuberal and late prepuberal). Measurements were made with 10 x micrometer lens and 10 x objective lens and the images were analyzed by means of the software for image capture (Motic Image Plus 2.0 ML, Media Cybernetics). In the same section where TD was obtained, we measured the height of seminiferous epithelium, considering from the basal membrane to the luminal border, by measuring the height of the seminiferous epithelium in four different parts of each tubule and then calculating the mean value. The procedure was the same used for determining TD, except with the use of a 40 x objective lens.

2.4 Statistical methods

Data were expressed as mean + s.d. values. Parametric data (body mass and testicular length) were analyzed by ANOVA followed by Tukey's test ($p < 0.05$), and non-parametric data (testicular mass, width, and thickness) were analyzed by Kruskal-Wallis' test followed by t-test ($p < 0.01$). Simple correlation coefficient (r) was determined among the numerical variables. All statistical processing was conducted under the computational support of ASSISTAT 7.6 beta.

3 Results

Changes in body mass during postnatal growth differed between weeks 3, 5, 6, and 9 ($p < 0.05$; Figure 1). Testicular morphometry did not differ ($p > 0.05$) between the right and left antimeres, thus, data of the right antimere were presented. The testis mass showed a discontinuous and dramatic growth (about 38.0-fold) up to 10 weeks, particularly at 3 (6.0-fold), 5 (9.3-fold), 6 (19.3-fold), 9 (27.0-fold) and 10 (38.0-fold) weeks of age, not presenting a trend towards stabilization (Figure 2a). The testis length and width showed a discontinuous growth (~ 3.6-fold) up to 10 weeks, particularly at 3 (~ 1.6-fold), 5 (~ 2.2-fold), 6 (~ 3.0-fold) and 10 (~ 3.5-fold) weeks of age (Figure 2 b,c). Exception was the thickness, which showed a continuous growth up to 9 weeks (3.3-fold), particularly at 3 (2.0-fold) and 5 (3.2-fold) weeks of age (Figure 2d).

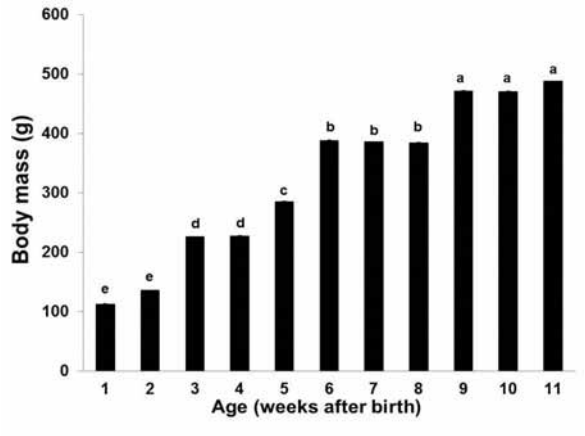

Figure 1. Changes in body mass from 1 to 11 weeks after birth in *Cavia porcellus* (mean + s.d.). Different letters at the different ages investigated indicate statistical significance at the level of $P < 0.05$.

The gonadosomatic index increased (~ 8.0-fold) up to 10 weeks, ranging from 0.06 to 0.48%, revealing an unstable growth with statistically significant differences ($p < 0.05$) at weeks 3 (0.16%), 6 (0.29%), 9 (0.35%), and 10 (0.48%) and a significant decrease at week 4 (0.10%) of age (Figure 3), which coincided with the changes observed in mass, length and width testicular (Figure 2 a,b,c).

Very high correlation ($p < 0.01$) was observed between gonadosomatic index and testicular mass at weeks 3 ($r = 0.98$), 4 ($r = 0.99$), 7 ($r = 0.99$), 8 ($r = 0.96$), 9 ($r = 0.98$), and 10 ($r = 0.99$), and high correlation ($p < 0.05$) at 5 weeks ($r = 0.90$) and 6 ($r = 0.95$). On the other hand, correlation between gonadosomatic index and body mass was observed only at week 10 ($r = 0.90$; $p < 0.05$).

During postnatal testicular development, we study the cell populations present in the seminiferous epithelium, in an attempt to characterize the first spermatogenic wave and establishment of puberty. Important changes were observed in the predominant cell type of seminiferous tubules from week 1 to week 11 and clear differences were seen between weeks with regards to the cell types predominant in the seminiferous tubule. At the

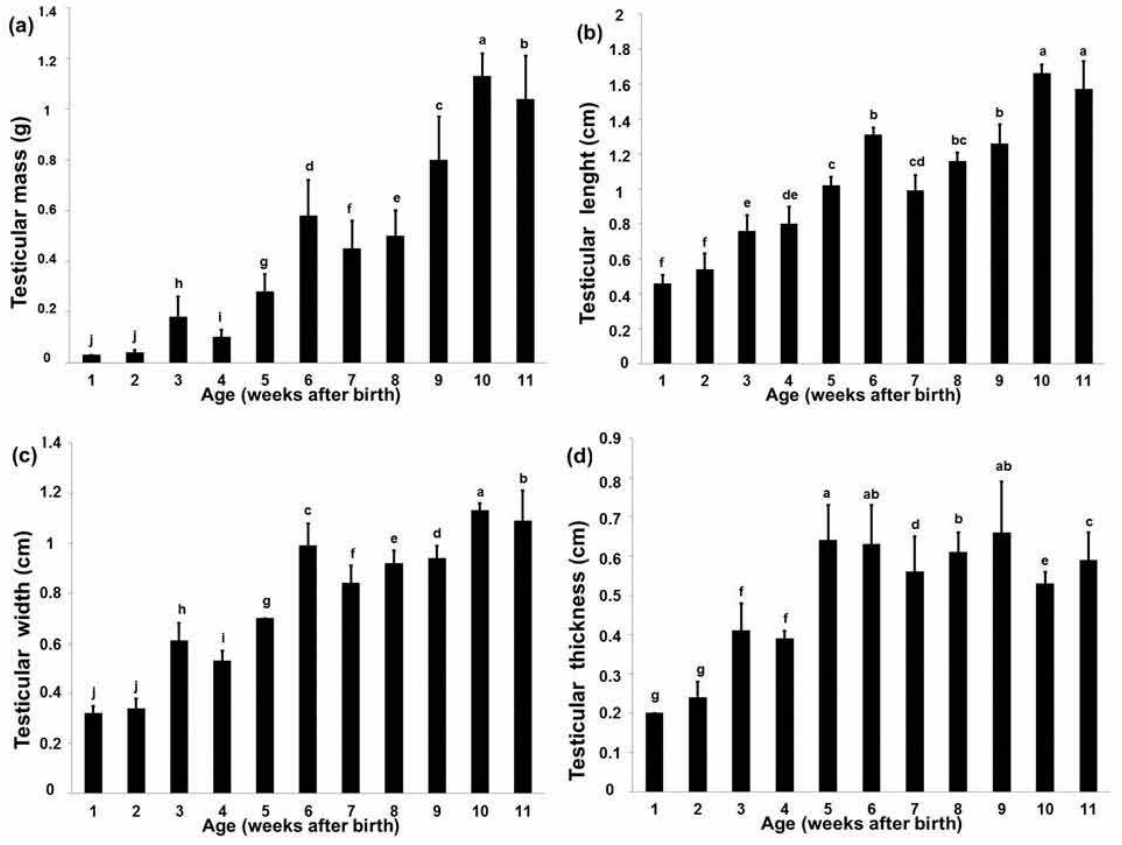

Figure 2. Testis biometric data from 1 to 11 weeks after birth in guinea pigs (*Cavia porcellus*) (mean ± s.d.). Different letters at the different ages investigated indicate statistical significance at the level of $P < 0.05$.

earliest time examined, at week 1, we observed the presence of solid seminiferous cords showing the presence of immature Sertoli cells, spermatogonia and gonocytes. Gonocytes were in contact with the basal lamina and the spermatogonies formed a monolayer, arranging themselves into rows located further away from it (Figure 4a). At 2 weeks, the seminiferous cords showed the onset of the lumen formation and spermatogenic activity, with the first spermatogonies type A close to the basal membrane and the emergence of the first primary spermatocytes at preleptotene/leptotene located close to the basal membrane and the primary spermatocytes at pachytene, a little further away from it, appeared at 3 weeks. In the beginning, gaps had variable size, many solid seminiferous cords persisted, and gonocytes were no longer observed and the Sertoli cells were more differentiated (Figure 4b). At 4 weeks, we did not observe new cell types, while at week 5 the first round spermatids emerged, forming 2 or 3 layers, mitosis figures and differentiated Sertoli cells (Figure 4c). Drastic seminiferous tubule changes were observed at 6 weeks of age. Also, the presence of a patent lumen, differentiated Sertoli cells, and all kinds of germ cells, including mature elongated spermatids and spermatozoa released into the lumen were observed (Figure 4d). A similar histological pattern was noted at 7 and 8 weeks of age, and at the later ages investigated. At 9 weeks of age there was an expansion of the tubular lumen and cell proliferation in the seminiferous epithelium with another expansion of tubular diameter and the height of the seminiferous epithelium (Figure 4e), but no new cell types emerged. These findings persisted until 10 weeks of age. At 11 weeks of age, the seminiferous tubules looked like those at 6 to 10 weeks of age, however, we observed large amount of primary spermatocytes

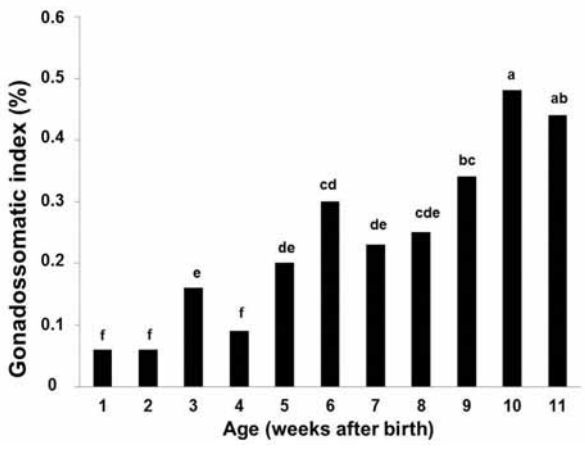

Figure 3. Gonadosomatic index (%) from 1 to 11 weeks after birth in guinea pigs (*Cavia porcellus*) (mean ± s.d.). Different letters at the different ages investigated indicate statistical significance at the level of $P < 0.05$.

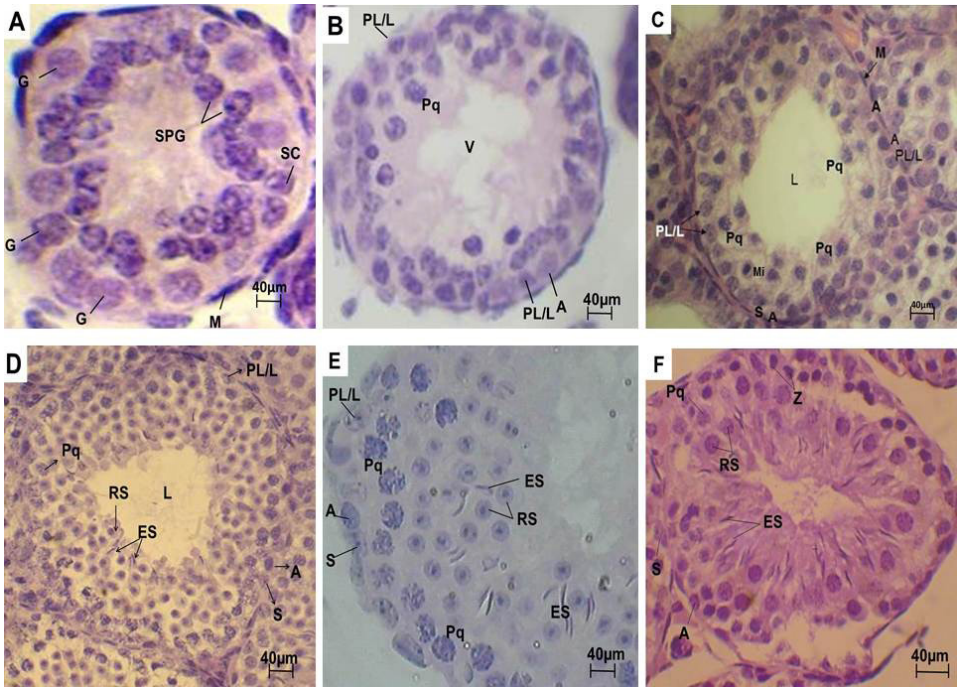

Figure 4. Photomicrograph of seminiferous cord or tubule cross-sections in *Cavia porcellus*, during postnatal development. (A) Seminiferous cord at one week of age showing the presence of myoid cells (M), immature Sertoli cells (SC), spermatogonia (SPG) and gonocytes (G); (B) Seminiferous cord at three weeks of age showing the beginning of the lumen formation (V) and the presence of spermatogonia type A (A), primary spermatocytes in preleptotene/leptotene (PL/L) and primary spermatocytes in pachytene (Pq); (C) Seminiferous cord at five weeks of age showing the presence of mature Sertoli cell (S), spermatogonia type A (A), preleptotene/leptotene (PL/L) and pachytene (Pq) spermatocytes and mitosis figures (Mi); (D) Seminiferous tubule in guinea pig at six weeks of age. Observe the presence of mature Sertoli cell (SC), pachytene spermatocyte (Pq), round spermatid (RS), elongated spermatid (ES) and the presence of evident lumen (L); (E) Seminiferous tubules at nine weeks of age. Note the presence of Sertoli cells (SC), pre-leptotene/ leptotene (PL/L) and pachytene (Pq) spermatocytes, round spermatids (RS) and elongated spermatids (ES); (F) Seminiferous tubule in guinea pig at eleven weeks of age, showing the same cell types indicated in panel and primary spermatocytes in zygotene (Z). Bar 40µm.

Table 1. Simple correlation coefficient (r) between age, tubular diameter (TD), seminiferous epithelium height (SEH), body mass and testicular biometry during postnatal development in *Cavia porcellus*.

	TD	SEH	BM	TM	TL	LW	TT
Age	0.90**	0.94**	0.97**	0.95**	0.94**	0.94**	0.78**
TD	------	0.97**	0.95**	0.86**	0.89**	0.93**	0.87**
SEH	------	------	0.96**	0.93**	0.91**	0.94**	0.78**

**$P < 0.01$; BM: body mass; TM: testis mass, TL: testis length; TW: testis width and TT: testis thickness.

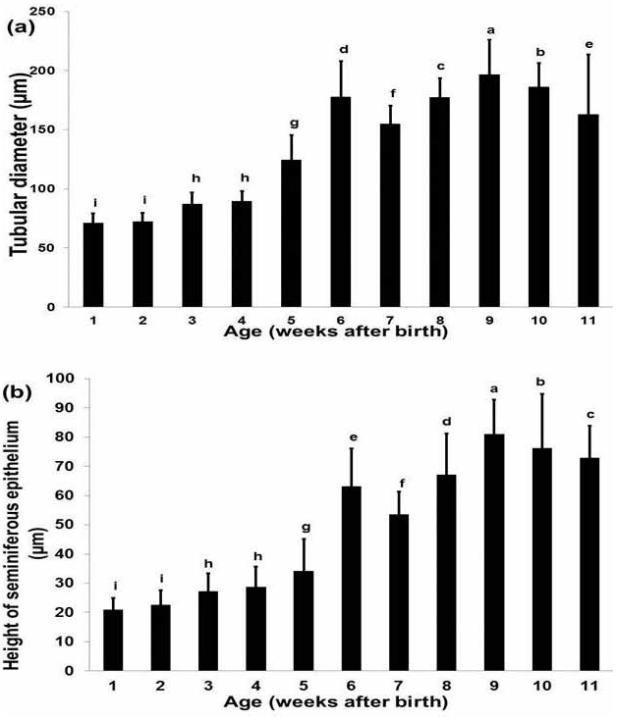

Figure 5. Diameter of cords and/or seminiferous tubules (µm) (a) and the seminiferous epithelium height (µm) (b) from 1 to 11 weeks after birth in guinea pigs (*Cavia porcellus*) (mean ± s.d.). Different letters at the different ages investigated indicate statistical significance at the level of $P < 0.05$.

at zygotene and decrease in the tubular diameter and height of the seminiferous epithelium (Figure 4f). We estimated, based on the changes described above, the time taken to undergo the transition between several stages, namely from spermatocyte (i.e. the beginning of meiosis) to round spermatids (the end of meiosis), and elongating and elongated spermatids (i.e. the completion of spermiogenesis), and the overall transition from spermatocytes to elongated spermatids. We found a transition time from spermatocytes to elongated spermatids of 21 days.

Tubular diameter and height of the seminiferous cords or tubules was similar ($P > 0.05$) from 1 to 2 weeks of age. A continuous growth was observed in these parameters thereafter, until the animals reached 6 weeks of age (~ 2.5 and 3-fold, respectively), and then increased again at 9 weeks of age (~ 2.8 and ~3.9-fold) reaching values of 196.60 µm (Figure 5a) for the tubular diameter and of 80.90 µm (Figure 5b) for the seminiferous epithelium height, and decreasing after this age.

Very high correlation ($p < 0.01$) was observed between the tubular diameter, seminiferous epithelium height, age, body mass, testicular mass, testicular length, testicular width and testicular thickness (Table 1).

4 Discussion

This study reported the results of a careful morphometric investigation of the body and testis during postnatal development and testicular differentiation in guinea pigs. In addition to providing valuable data regarding the reproductive biology of this species, our results may allow important comparisons between others rodents (agoutis, capybaras, mice, rats and chinchillas) and others mammal species. The parameters investigated, such as testis mass, tubular diameter, the first release of sperm from the seminiferous epithelium and the morphological characteristics of spermatogenic cells of the germinal epithelium, enabled the classification of the following phases of testicular development in guinea pigs: impuberal (weeks 1), early prepuberal (weeks 2 to 4), late prepuberal (week 5), puberal (weeks 6 to 8), postpuberal 1 (weeks 9 and 10), and postpuberal 2 (week 11).

Body mass and testicular mass showed, respectively, four and five quick growth points: the first in the early prepuberal phase, the second in the late prepuberal phase, the third in the puberal phase, the fourth, in both body and testicular mass, in the postpuberal 1 phase, and the fifth, only in testis mass, in the postpuberal 1 phase. These periods partially disagree with other domestic mammal species (COUROT, HOCHEREAUA-DE REVIERS and ORTAVANT, 1970) and rodents (COUROT, HOCHEREAUA-DE REVIERS and ORTAVANT, 1970; ASSIS NETO, CARVALHO, MELO et al., 2003a), which had periods of increased growth, coinciding with the phases impuberal, prepuberal, and puberal of sexual development. The acceleration in the testicular growth curve observed after puberty in guinea pigs was not so different from that of the common agouti (ASSIS NETO, MELO, CARVALHO et al., 2003b).

The quick growth of testicular mass, particularly observed in the pubertal phase, when the entire seminiferous epithelium was already formed, coincided with the largest expansion of tubular diameter and height of the seminiferous epithelium and with the presence of spermatozoa in the tubular lumen. The high correlation between these parameters indicated that testicular mass was a quantitative indicator of sperm production by guinea pigs and, therefore, testicular mass may constitute a useful tool for evaluating the sperm production capacity of this species, as described by other authors (FRANÇA and RUSSELL, 1998; JOHNSON, VARNER, ROBERTS et al., 2000). After puberty, testicular development in guinea pigs was similar to that in other mammal and rodent species (COUROT, HOCHEREAUA-DE REVIERS and ORTAVANT, 1970; ASSIS NETO, CARVALHO, MELO et al., 2003a). Highly significant correlation ($p < 0.01$) between body mass, age and other testicular parameters agreed with Murrah buffalo bulls (LUZ, SANTOS, ANDRIGHETTO et al., 2013) and contrasted to the correlation described only between the testis mass and the length, width and tubule diameter in collared

peccary (GUIMARÃES, CARDOSO, FERREIRA et al., 2013) and between testicular mass and length and between testicular mass and width (FRANÇA, SILVA-JUNIOR, CHIARINI-GARCIA et al., 2000; FERREIRA, GUIMARÃES, LUZ-RAMOS et al., 2004; SONNER, MIGLINO, SANTOS et al., 2004; OHASHI, MIRANDA, CORDEIRO et al., 2007). Thus, highly significant correlation ($p < 0.01$) between age, body mass and testicular biometric data indicated that, in guinea pigs, testicular development depended both on age and body development, as in other rodent species (ASSIS NETO, CARVALHO, MELO et al., 2003a; FERREIRA, GUIMARÃES, LUZ-RAMOS et al., 2004), in opposition to other mammal species, in which testicular development rather depends on body development (COUROT, HOCHEREAUA-DE REVIERS and ORTAVANT, 1970) or those with gradual loss in the correlations between testicular measurements and body mass in the adult phase (SOUZA, ARAÚJO, OLIVEIRA et al., 2010).

GSI in guinea pigs was within the range regarded as normal for rodents, 0.10% to 8.00% (KENAGY and TROMBULAK, 1986), and the value observed in the phase postpuberal 2 (0.44%) was inferior to that observed in Swiss mice, 0.69% (MORAIS, BARBOSA, NEVES et al., 2009), and chinchillas, 0.80% (LEAL and FRANÇA, 2008), and superior to those observed in pacas, 0.22%, agoutis, 0.33% (COSTA, LEAL, FERREIRA et al., 2013), and capybaras (0.14% in MOREIRA, CLARKE and MACDONALD, 1997; 0.12% in PAULA, COSTA and MATTA, 2002). These findings corroborate the observation that the larger the animal, the lower the body mass allocated in testes (CALDEIRA, PAULA, MATTA et al., 2010). The evolution of GSI showed consistency with the variations in testicular mass (Figurre 3), so that a significant correlation was observed between these parameters from the early prepuberal phase until the postpuberal 1 phase, indicating that at these weeks the increase in testicular mass followed the increase observed in body mass and that this index reflected reproductive activity better than body mass, as this was significantly correlated to GSI only at the postpuberal 1 phase. Therefore, except in this phase, body mass does not constitute a good indicator of weekly variations in reproductive organs, corroborating other authors (ORÓSTEGUI, PARRAGUEZ, ADARO et al., 2000; CEPEDA, ADARO and PEÑAILILLO, 2006).

The establishment of spermatogenesis is a long and progressive phenomenon in which several phases can be distinguished after birth (COUROT, HOCHEREAUA-DE REVIERS and ORTAVANT, 1970; FRANÇA and CARDOSO, 1988). Analyses of histological sections of testicular parenchyma are important, as they indicate how the spermatogenic process is associated to the growth of gonads. Evaluating the testicular parenchyma composition in guinea pigs, we observed that findings in the impuberal phase corresponded those described in common agoutis (ASSIS NETO, MELO, CARVALHO et al., 2003b), chinchillas (LEAL and FRANÇA, 2008) and other mammal species (FRANÇA and CARDOSO, 1988; MURTA, COSTA, SANTOS et al., 2009); they differed, however, from Ferreira, Guimarães and Luz-Ramos et al. (2004), who observed the disappearance of gonocytes. On the other hand, the location of gonocytes, close to basal membrane, and that of spermatogonies, in monolayers more distant from the basal membrane, differed from that in wild boars (MURTA, COSTA, SANTOS et al., 2009) and chinchillas (LEAL and FRANÇA, 2008), which show a distribution pattern inverse to that of guinea pigs. The duration of the impuberal phase, around 7 days, was slightly lower than the 10 days described for hamsters and guinea pigs and 2 months for Piau boars (FRANÇA, SANTANA-CASTRO and CARDOSO, 1988); 20 days for *Mus spicilegus* (MONTOTO, ARREGUI, SÁNCHEZ et al., 2012); wild boars (MURTA, COSTA, SANTOS et al., 2009) and chinchillas (LEAL and FRANÇA, 2008), as well as 5 months for common agoutis (ASSIS NETO, MELO, CARVALHO et al., 2003b).

The onset of lumen formation in guinea pigs characterized the early prepuberal phase that took place earlier (at week 2) than in other mammal (FRANÇA, SILVA-JUNIOR, CHIARINI-GARCIA et al., 2000; MURTA, COSTA, SANTOS et al., 2009) and rodent species (ASSIS NETO, MELO, CARVALHO et al., 2003b; LEAL and FRANÇA, 2008). The gaps with varying sizes occurred alongside many solid testicular cords, indicating asynchrony in the development of testicular parenchyma described earlier (FRANÇA and CARDOSO, 1988; ASSIS NETO, MELO, CARVALHO et al., 2003b; MURTA, COSTA, SANTOS et al., 2009). The early prepuberal phase was also characterized by the disappearance of gonocytes and the start of spermatogenic activity, with the emergence of the first spermatogonia A, primary spermatocytes, and the support cells already differentiated and having features of Sertoli cells. The disappearance of gonocytes seems to occur due to continued proliferation and differentiation of this cell type in spermatogonia (AGUIAR, ARAÚJO and MOURA, 2006). In guinea pigs, the emergence of spermatogonia A and primary spermatocytes took place earlier than in other mammal (FRANÇA, SANTANA-CASTRO and CARDOSO, 1988; FRANÇA and CARDOSO, 1988; MURTA, COSTA, SANTOS et al., 2009) and rodent species (ASSIS NETO, MELO, CARVALHO et al., 2003b; FERREIRA, GUIMARÃES, LUZ-RAMOS et al., 2004; LEAL and FRANÇA, 2008; MONTOTO, ARREGUI, SÁNCHEZ et al., 2012) and later than in *Mus musculus* and *Mus spicilegus* (MONTOTO, ARREGUI, SÁNCHEZ et al., 2012). In the late prepuberal phase, the first round spermatids emerged, forming 2 or 3 layers, as well as mitotic figures, corroborating other authors (ASSIS NETO, MELO, CARVALHO et al., 2003b). The timing of appearance of sperm cells in this study was similar to that described by Rodriguez-Casuriaga, Geisinger, and Santinaque et al. (2011) to *C. porcellus*, in which the establishment of the first spermatogenic wave was followed by flow cytometry, and confirmed by microscopical observations of cross sections of seminiferous cords / tubules.

The transition time from spermatocytes to elongated spermatids in guinea pig was lower than to the 40, 36, 31 and 25 days described, respectively, for *Mus musculus, Mus pahari, Mus spretus* and *Mus spicilegus* (MONTOTO, ARREGUI, SÁNCHEZ et al., 2012). According this author, species with lower levels of sperm competition (*Mus pahari* and *Mus musculus*) has a much slower transition, i.e. more days taken to move along the cell stages (about 35–40 days) and species with higher sperm competition levels (*Mus spretus* and *Mus spicilegus*) had a much faster transition time (about 31 and 25 days, respectively). Our results also agree with others that species with higher sperm competition levels have higher absolute and relative testes mass, have shorter cycles of the seminiferous epithelium (indicative of a shorter duration of spermatogenesis), thus allowing for an increase in sperm production rate (RAMM and STOCKLEY, 2010; MONTOTO, ARREGUI, SÁNCHEZ et al., 2012). These

early differences in testicular architecture and kinetics of sperm formation appear to be responsible for the differences observed at sexual maturity, which influence sperm production rates.

The identification of the first spermatozoa in the tubular lumen characterizes complete spermatogenesis and it may determine the puberty age in a species (COUROT, HOCHEREAUA-DE REVIERS and ORTAVANT, 1970). In this study, this took place at week 6, i.e., at 42 days of life, less than the 60 days previously reported for this species (ASSIS NETO, CARVALHO, MELO et al., 2003a). Our results were lower than the 45-105 days in Spix's yellow-toothed cavy (SANTOS et al., 2012), 90 days reported in chinchilla (LEAL and FRANÇA, 2008) and 9 to 10 months in agoutis (ASSIS NETO, MELO, CARVALHO et al., 2003b). In guinea pig, the puberal phase was also characterized by the proliferation of spermatogenic cells, increased testicular mass and accelerated weight gain, as observed in common agouti (ASSIS NETO, MELO, CARVALHO et al., 2003b), and the testicular parenchyma composition did not strongly disagree with other mammal and rodent species (ASSIS NETO, CARVALHO, MELO et al., 2003c; FERREIRA, GUIMARÃES, LUZ-RAMOS et al., 2004; MOURA, GUERRA, SILVA et al., 2006; LEAL and FRANÇA, 2008; MURTA, COSTA, SANTOS et al., 2009).

In the phase postpuberal 1, the structure of seminiferous tubules was similar to that of the puberty phase, however, the increased number of spermatogenic cells was noticeable, reflecting an improved intrinsic effectiveness of spermatogenesis in agreement with others (COUROT, HOCHEREAUA-DE REVIERS and ORTAVANT, 1970; FRANÇA and CARDOSO, 1988; MURTA, COSTA, SANTOS et al., 2009). In the phase postpuberal 2, seminiferous tubules had an aspect similar to that of the phases puberal and postpuberal 1, however, there was no increase in the height of germinal epithelium, demonstrating an epithelium stability that did not disagree with that of wild boars (MURTA, COSTA, SANTOS et al., 2009).

Testicular morphometric analyses are important in describing the spermatogenic process in each species; hence, in research involving the testicular function, measuring tubular diameter and the height of seminiferous epithelium constitutes an approach classically adopted to indicate the spermatogenic activity (FRANÇA and RUSSELL, 1998; PAULA, FRANÇA and CHIARINI-GARCIA, 1999; MOURA, GUERRA, SILVA et al., 2006). Tubular diameter and the height of seminiferous epithelium in guinea pigs differed between the phases of testicular development, and acceleration in the growth rhythm of both coincided with the full installation of spermatogenesis at puberty. Correlation between tubular diameter and testicular growth was also positive in common agoutis (FERREIRA, GUIMARÃES, LUZ-RAMOS et al., 2004), while in capybaras there was negative correlation between age and tubular diameter (MOREIRA, CLARKE and MACDONALD, 1997). Although the tubular diameter remains relatively constant in a sexually mature animal, it can present significant differences across several species (FRANÇA and RUSSELL, 1998). The growth curve of tubular diameter in guinea pigs resembled that of agoutis and it disagreed with the decrease in the puberal phase cited in guinea pigs by Assis Neto, Melo and Carvalho et al. (2003d), while the increase ($p < 0.05$) after puberty was observed in agoutis (ASSIS NETO; MELO; CARVALHO et al., 2003d) and chinchillas (LEAL and FRANÇA, 2008). The values found for seminiferous tubule diameter in pubertal-guinea pigs and in postpuberal 1 guinea pigs was within the range observed in most mammal species, from 180 to 300 µm (FRANÇA and RUSSELL, 1998), being similar to that of adult Swiss mice (MORAIS, BARBOSA, NEVES et al., 2009) and chinchillas (LEAL and FRANÇA, 2008), higher than aged mice (MEHRAEIN and NEGAHDAR, 2011), and lower than Wistar rats (ALMEIDA, WEISS, CASTRO et al., 2000), rats (DAMODAR, D'SOUZA, BISWAS et al., 2012), lowland pacas (CARRETTA JÚNIOR, 2008), and capybaras (PAULA, COSTA and MATTA, 2002; PAULA and WALKER 2013). The values found for seminiferous epithelium height was within the range observed in domestic species, 60 to 100 µm (FRANÇA and RUSSELL, 1998) since the puberal phase, being higher than chinchilla (LEAL and FRANÇA, 2008) at puberty. In the phase postpuberal 1, our results were higher than those in adult Swiss mice (MORAIS, BARBOSA, NEVES et al., 2009), and lowland pacas (CARRETTA JÚNIOR, 2008), similar to that in adult capybaras (PAULA and WALKER, 2013), chinchillas (LEAL and FRANÇA, 2008) and aged mice (MEHRAEIN and NEGAHDAR, 2011) and lower than that in rats (DAMODAR, D'SOUZA, BISWAS et al., 2012).

In conclusion, this study shows the early differences in testicular architecture and kinetics of sperm formation, indicating that the testicular development, timing of transition between the appearance of a majority of tubules containing spermatocytes to a majority of tubules with elongated spermatids and puberty are faster than in other mammal and rodent species, demonstrating the suitability of the guinea pig testes as a model for studies of the testicular development and spermatogenesis.

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