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Testis Morphometry, Duration of Spermatogenesis, and Spermatogenic Efficiency in the Wild Boar (*Sus scrofa scrofa*)[1]

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ABSTRACT

The wild boar is a natural inhabitant of Europe, Asia, and North Africa and is phylogenetically the ancestor of the domestic pig. Because of its phylogenetic and economic importance, this species is an interesting model for studying testis function in boars. Therefore, the present study was performed to investigate the testis structure, spermatogenic cycle length, and Sertoli cell (SC) and spermatogenic efficiencies in eight adult wild boars. Each spermatogenic cycle lasted 9.05 days, and the total duration of spermatogenesis was estimated as lasting approximately 41 days. The percentages of testis volume occupied by seminiferous tubules and by Leydig cells were 87% and 6%, respectively. The mean number of SCs per gram of testis was 42 million. The SC (round spermatids per SC) and spermatogenic (daily sperm production per gram of testis) efficiencies were 6.6 cells and 28.6 million, respectively. In general, the testis structure, overall germ cell associations at the different stages of the seminiferous epithelium cycle, and duration of spermatogenesis in the wild boar were similar to those in domestic pigs. Probably because of the small size of Leydig cells (~400 μm^3), their number per gram of testis (157 million) was the highest among investigated mammalian species. Although the SC efficiency in wild boars was low, their spermatogenic efficiency was comparable to that observed in domestic pigs, mainly because of the higher number of SCs per gram of testis in wild boars. These data suggest that SCs became more efficient during evolution, genetic selection, and domestication in pigs.

Leydig cells, male reproductive tract, Sertoli cells, spermatogenesis, testis

INTRODUCTION

The wild boar (*Sus scrofa scrofa*) is a swine originating from Europe, Asia, and North Africa [1] and is now found on all continents, having being economically exploited because of the high commercial value of its meat. Mitochondrial DNA investigations showed that the wild boar is the ancestor of the domestic pig (*Sus scrofa domesticus*) [2]. These two species diverged almost 500 000 years ago, much earlier than the pig domestication that took place approximately 9000 years ago [2]. Although the wild boar represents an attractive model to study testis function in boars, there are few data regarding the reproductive biology of this species [3, 4].

Spermatogenesis is a cyclic and highly coordinated process that begins with mitotic spermatogonial proliferation, proceeds through two meiotic divisions, and is followed by spermiogenesis in which haploid spermatids develop into spermatozoa [5]. In mammals, all types of germ cells are found in the germinal epithelium encompassing different cell associations called stages. The stages of the seminiferous epithelium cycle may be characterized according to changes in the shape of the spermatids nuclei, its arrangement within the germinal epithelium, and the occurrence of meiotic divisions [6–9] or may be based on the development of the acrosomic system and the morphology of developing spermatids nuclei [5, 10]. Besides being useful for comparative studies, accurate investigation and characterization of the spermatogenic process can provide answers to important questions about testis structure and function and about their correlations with physiological and biochemical findings [11, 12]. Also, quantitative investigation of the testis requires identification of the seminiferous epithelium cycle stages and knowledge of their duration [7, 12]. The total duration of spermatogenesis takes approximately 4.5 cycles and lasts approximately 30–75 days in the mammalian species already investigated [5, 12]. The spermatogenic cycle length has been generally considered to be constant for a given species [12–14], and investigations involving the spermatogonial transplantation from rats to mice have shown that the duration of spermatogenesis is under the control of the germ cell genotype [15].

Another important parameter in evaluating testis function is the determination of the Sertoli cell (SC) efficiency, which is the best indicator of spermatogenic efficiency (daily sperm production [DSP] per gram of testis). In a species-specific manner, each SC is able to support a limited number of germ cells, and the number of SCs per testis in mammals is believed to be established before puberty [12, 16, 17].

To our knowledge, no study has reported on the testis structure and function, particularly related to spermatogenesis, in the wild boar. The main objective of the present study was to perform an accurate histological and morphofunctional investigation of the testis and of the duration of the spermatogenic events in sexually mature wild boars. The results will allow a comparison with the data already obtained among domestic pigs.

MATERIALS AND METHODS

Animals

Eight sexually mature (approximately 12 mo old) wild boars (*Sus scrofa scrofa*) were utilized in the present study. All animals had phenotypic characteristics of the species. Species identity was confirmed by determining the number of chromosomes in lymphocyte cultures. In each of the animals examined, this number (n = 36) was characteristic of the wild boar, compared with 38 in the domestic pig. All samples were obtained in the first week of

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April, which is the beginning of autumn in the Southern Hemisphere. Sperm production in wild boars reared in the state of Minas Gerais, Brazil (latitude, 19° 55' S; longitude 43° 56' W), seems to be unaffected by the season, based on the yearlong birth of piglets; therefore, the examined samples were considered representative. Before castration, all animals received i.m. injections of 1 ml/20 kg of body weight of Stresnil (Johnson and Johnson) plus 1 ml/5 kg of body weight of Amplictil (Rhodia). All surgical procedures were performed by a veterinarian and followed approved guidelines for ethical treatment of animals.

Thymidine Injections and Tissue Preparation

To estimate the duration of the seminiferous epithelium cycle, all animals received intratesticular injections of tritiated thymidine (thymidine [methyl-^{3}H], specific activity 3.03 TBq/mmol, 82.0 Ci/mmol; Amersham Life Sciences), a specific marker for cells that are synthesizing DNA. The injections of 75 μCi of tritiated thymidine were performed in three different regions of each testis (near the epididymal tail) using a sterile hypodermic needle. Two animals were utilized for each interval considered (at 1 h and at 7, 14, and 21 days after thymidine injections). The testes were fixed by gravity-fed perfusion through the testicular artery with 0.9% saline containing 5000 IU of heparin/L for 5 min and, subsequently, with 4% buffered glutaraldehyde for 25 min. After fixation, testes were trimmed out from the epididymis, weighed, and cut longitudinally with a razor blade. Tissue samples measuring 2–3 mm in thickness were obtained near the site of thymidine injections. Testis fragments were routinely processed and embedded in plastic (glycol methacrylate).

For autoradiographic analysis, unstained testis sections (4 μm in thickness) were dipped in autoradiography emulsion (Kodak NTB-2; Eastman Kodak Company, Rochester, NY) at 45°C. After drying for approximately 1 h at 25°C, these sections were placed in sealed black boxes and were stored at 4°C for approximately 7 wk. Subsequently, the testis sections were developed in Kodak D-19 solution at 15°C [18] and were stained with toluidine blue. Analyses of these sections were performed by light microscopy to detect the most advanced germ cell type labeled at different periods after thymidine injections. Cells were considered labeled when 4–5 or more grains were present over the nucleus in the presence of low to moderate background.

Testis Morphometry

To perform light microscopic investigations, testis fragments were routinely processed and embedded in plastic. Subsequently, 4-μm-thick sections were obtained and stained with toluidine blue. The tubular diameter and height of the seminiferous tubule epithelium were measured at 100× magnification using an ocular micrometer calibrated with a stage micrometer. At least 20 tubular profiles that were round or nearly round were chosen randomly and measured for each animal. The volume densities of testis tissue components were determined using a 441-intersection grid placed in the ocular of the light microscope. Fifteen fields chosen randomly (6615 points) were scored for each animal at 400× magnification. Artifacts were rarely seen and were not included in the data. Points were classified as one of the following: seminiferous tubule (comprising tunica propria, epithelium, and lumen), Leydig cell, connective tissue, blood and lymphatic vessels. The volume of each testis component was determined as the product of the volume density and the testis volume. For subsequent morphometric calculations, the specific gravity of testis tissue was considered to be 1.0 [9, 19]. To obtain the testis volume, the mean value of the testis capsule plus mediastinum (~6% in boars) [20] was excluded from the testis weight. The total length of the seminiferous tubule (in meters) was obtained by dividing the seminiferous tubule volume by the squared radius of the tubule times π [21, 22].

Stages of the Spermatogenic Cycle and the Duration of Spermatogenesis

Stages of the cycle in wild boars were characterized based on the shape and location of spermatid nuclei, the presence of meiotic divisions, and the overall seminiferous epithelium composition. This method, known as the tubular morphology system [7], provides eight stages of the seminiferous epithelium cycle. The relative stage frequencies were determined from the analysis of 400 seminiferous tubule cross-sections per animal at 400× magnification. These tubules were chosen randomly, and both testes were analyzed for each animal (n = 7). The duration of each spermatogenic cycle was estimated based on the stage frequencies and the most advanced germ cell type labeled at different periods investigated after thymidine injections. The total duration of spermatogenesis comprised approximately 4.5 cycles that are necessary for this process to be completed, from type A spermatogonia to spermiation [14].

TABLE 1. Biometric and morphometric data in sexually mature wild boars.

Parameter (n = 7)[a]	Mean ± SEM
Body weight (kg)	39.7 ± 2.9
Testis weight (g)[b]	64.1 ± 11.0
Right testis	63.4 ± 11.0
Left testis	64.8 ± 11.0
Gonadosomatic index (%)	0.314 ± 0.04
Testis parenchyma volume density (%)	
Seminiferous tubule	86.9 ± 1.3
Tunica propria	2.1 ± 0.1
Seminiferous epithelium	73.8 ± 1.3
Lumen	11.0 ± 2.1
Intertubular compartment	13.1 ± 1.2
Leydig cell	6.3 ± 0.9
Connective tissue	5.2 ± 0.3
Blood vessels	1.4 ± 0.2
Lymphatic vessels	0.2 ± 0.04
Tubular diameter (μm)	251.0 ± 14.0
Seminiferous epithelium height (μm)	75.0 ± 4.0
Tubular length per gram of testis (m)	18.3 ± 1.9
Total tubular length per testis (m)	1022 ± 133

[a] Number of animals utilized = n.
[b] Right testis plus left testis divided by two.

Cell Counts

All germ cell nuclei and SC nucleoli present at stage 1 were counted in 10 round or nearly round seminiferous tubule cross-sections chosen randomly for each animal. In addition, newly formed round spermatids present at stage 5 were counted in 10 seminiferous tubule cross-sections. These counts were corrected for section thickness and for nucleus or nucleolus diameter according to the method by Abercrombie [23], as modified by Amann and Almquist [24]. For this purpose, 10 nuclei or nucleoli diameters were measured for each cell type analyzed per animal. Cell ratios were obtained from the corrected counts obtained at stage 1. The total number of SCs was determined from the corrected counts of SC nucleoli per seminiferous tubule cross-section, and the total length of seminiferous tubules as described by Hochereau-de Reviers and Lincoln [25]. The DSP per testis and per gram of testis (spermatogenic efficiency) was obtained according to the following formula: DSP = [total number of SCs per testis × ratio of round spermatids to SCs at stage 1 × stage 1 relative frequency (%)]/stage 1 duration (in days) [26].

The individual Leydig cell volume was obtained based on the nucleus volume and on the proportion between the nucleus and cytoplasm. To calculate the proportion between the nucleus and cytoplasm, a 441-point square lattice was placed over the sectioned material at 400× magnification. For each animal, 1000 points over Leydig cells were counted. Because the Leydig cell nucleus in wild boars is spherical, its nucleus volume was obtained based on the mean nuclear diameter. For this purpose, the diameters of 30 nuclei showing evident nucleolus were measured for each animal. Leydig cell nuclear volume was expressed in cubic micrometers and was obtained by the formula $4/3\pi R^3$, where R = nuclear diameter/2. The number of Leydig cells per testis was estimated from the individual Leydig cell volume and from the volume occupied by Leydig cells in the testis parenchyma.

Statistical Analysis

All data are presented as the mean ± SEM. Student t-test and analysis of correlation were performed using STATISTICA 3.11 for Windows (StatSoft, Inc., Tulsa, OK). $P < 0.05$ was considered significant.

RESULTS

Biometric Data and Testis Volume Density

The mean testis weight in wild boars was approximately 64 g, with a gonadosomatic index (GSI) (testes mass divided by body weight) of approximately 0.3% (Table 1). The mean weights for the left and right testes were similar ($P > 0.05$), and a significant and positive correlation was observed between testis weight and body weight ($r = 0.87$). The volume densities of seminiferous tubules and Leydig cells were

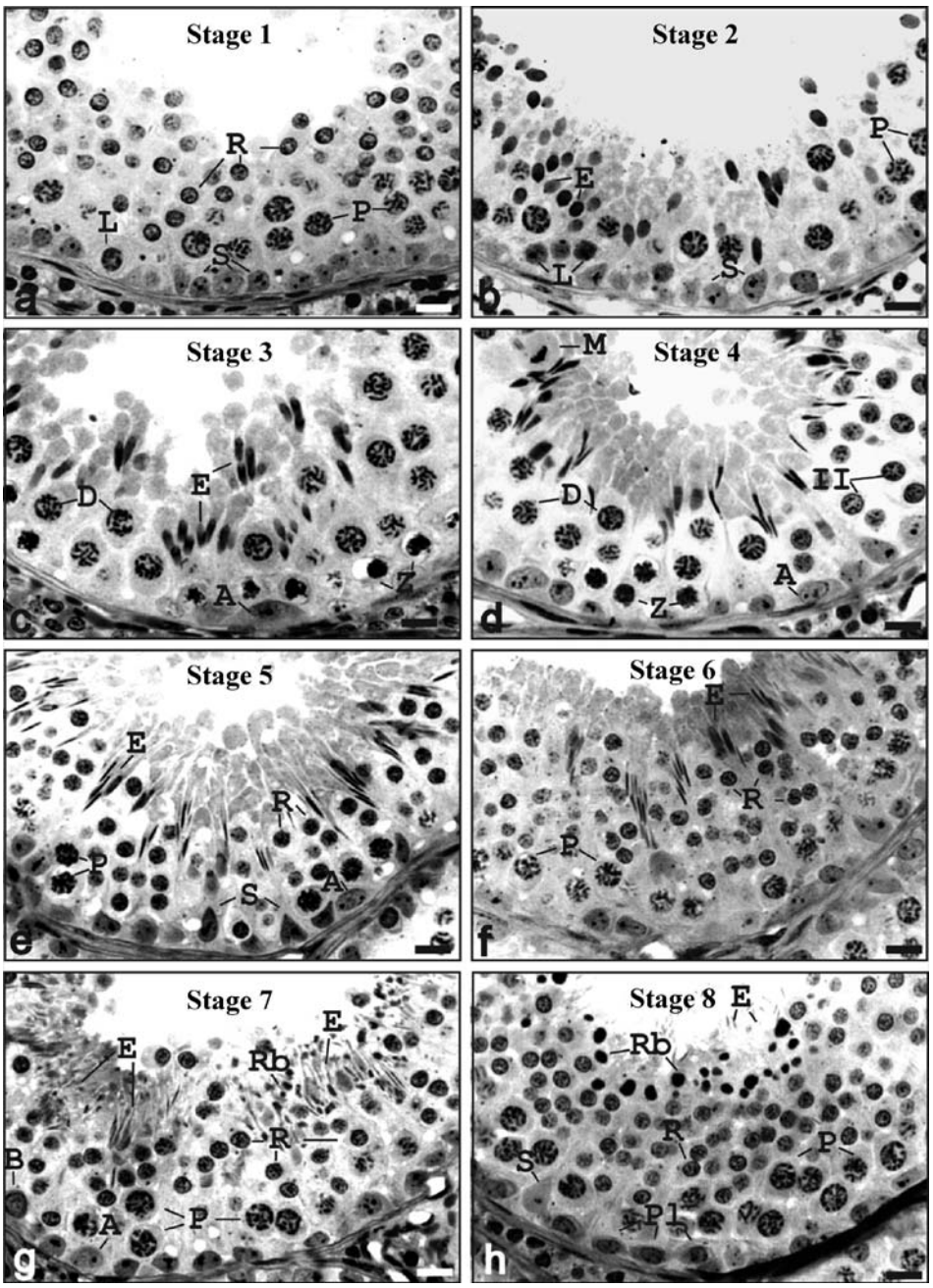

FIG 1. Stages 1 to 8 of the seminiferous epithelium cycle based on the tubular morphology system. **a**) Stage 1 shows leptotene spermatocytes (L), pachytene spermatocytes (P), round spermatids (R), and SCs (S). **b**) Stage 2 presents leptotene spermatocytes (L), pachytene spermatocytes (P), elongating spermatids (E), and SCs (S). **c**) Stage 3 contains type A spermatogonia (A), zygotene spermatocytes (Z), diplotene spermatocytes (D), and elongated spermatids (E). **d**) Stage 4 predominantly shows cells in the diplotene phase of meiosis (D), zygotene spermatocytes (Z), meiotic figures (M), secondary spermatocytes (II), and type A spermatogonia (A). **e**) Stage 5 contains type A spermatogonia (A), pachytene spermatocytes (P), newly formed round spermatids (R), elongated spermatids (E), and SCs (S). **f**) Stage 6 presents pachytene spermatocytes (P), round spermatids (R), and elongated spermatids (E). **g**) Stage 7 shows types A and B spermatogonia (B), pachytene spermatocytes (P), round spermatids (R), elongated spermatids (E), and residual bodies (Rb). **h**) Stage 8 shows pachytene spermatocytes (P), round spermatids (R), elongated spermatids (E), SCs (S), and residual bodies (Rb). Bar = 15 μm.

approximately 87% and 6%, respectively (Table 1). Therefore, Leydig cells occupy 50% of the intertubular compartment. The mean tubular diameter and epithelial height were 251 μm and 75 μm, respectively (Table 1). Based on the volumes of the testis parenchyma (testis weight minus [tunica albuginea plus mediastinum weight]) and the seminiferous tubules and on the mean tubular diameter, the mean seminiferous tubular length per gram of testis was 18 m, and the mean total seminiferous tubular length per testis was 1022 m (Table 1).

Stages of the Seminiferous Epithelium Cycle and Relative Stage Frequencies

The eight stages of the seminiferous epithelium cycle in wild boars are briefly described in the legend to Figure 1. The mean ± SEM percentages of each stage were as follows: stage 1, 8.2% ± 0.5%; stage 2, 10.2% ± 0.7%; stage 3, 16.9% ± 0.6%; stage 4, 14.4% ± 0.7%; stage 5, 13.0% ± 0.8%; stage 6, 17.7% ± 0.6%; stage 7, 11.5% ± 0.6%; and stage 8, 8.1% ± 0.6%. Stage 6 had the highest frequency, and stages 1 and 8 had the lowest frequencies. The premeiotic (stages 1–3) and postmeiotic (stage 5–8) stages occupied approximately one third and one half of the total stage frequencies, respectively.

Seminiferous Epithelium Cycle Length

The most advanced germ cell types labeled at different periods investigated after thymidine injections are given in Table 2 and in Figure 2. At approximately 1 h after injection, the most advanced germ cell types labeled were preleptotene spermatocytes or cells in transition from preleptotene to leptotene. These cells were present at the end of stage 2 and were located in the basal compartment (Fig. 2a). The most advanced germ cell types labeled at 7 days after thymidine injection were pachytene spermatocytes**,** present at the end of stage 8 (Fig. 2b). Labeled round spermatids at stage 6 were

TABLE 2. The length (days) of seminiferous epithelium cycle (X ± SEM).[a]

Time after injection	Most advanced germ cell type labeled[b]	Stage of the cycle	No. of cycles traversed	Cycle length based on labeling in preleptotene/leptotene	Cycle length based on intermediate labeling point
1 h	Pl/L	2	—	—	—
7.139 days[c]	P	8	0.816	8.75	—
14.097 days[c]	R	6	1.620	8.72	—
20.972 days[c]	E	4	2.319	9.05	—
6.958 days[d]	—	—	0.804	—	8.65
13.833 days[e]	—	—	1.497	—	9.24
6.875 days[f]	—	—	0.693	—	9.92

[a] Mean duration of the cycle based on P1/L and intermediate labeling = 9.05 ± 0.18 days.
[b] Pl/L, preleptotene/leptotene primary spermatocytes; P, pachytene primary spermatocytes; R, round spermatids; E, elongating spermatids.
[c] Total time after thymidine injection minus 60 min.
[d] Total time between 7 and 14 days.
[e] Total time between 7 and 21 days.
[f] Total time between 14 and 21 days.

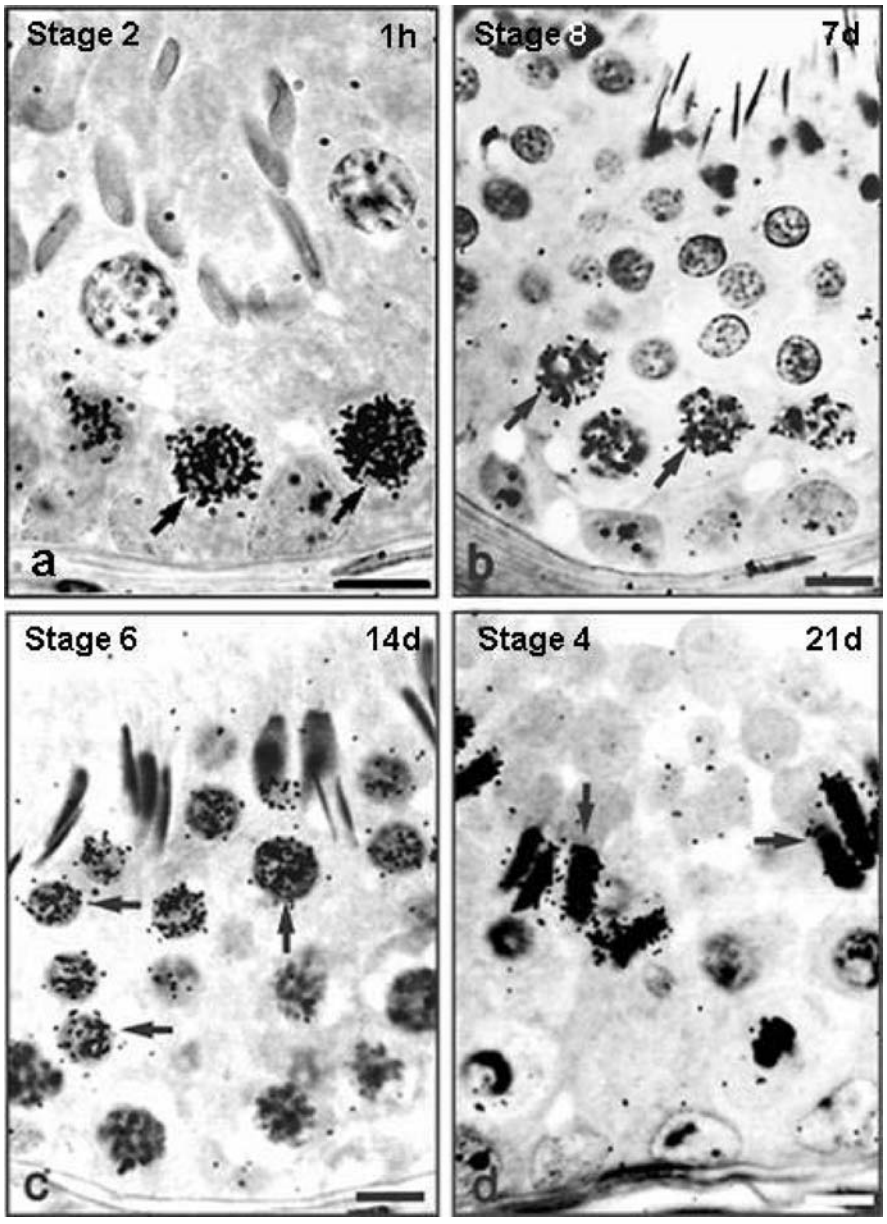

FIG 2. The most advanced germ cell types labeled after different periods following intratesticular injections of tritiated thymidine were as follows: **a**) At 1 h after injection, leptotene primary spermatocytes (arrows) at the end of stage 2. **b**) At 7 days after injection, pachytene spermatocytes (arrows) at stage 8. **c**) At 14 days after injection, round spermatids (arrows) at stage 6. **d**) At 21 days after injection, elongated spermatids at stage 4. Bars = 8 μm (**a** and **c**) and 10 μm (**b** and **d**).

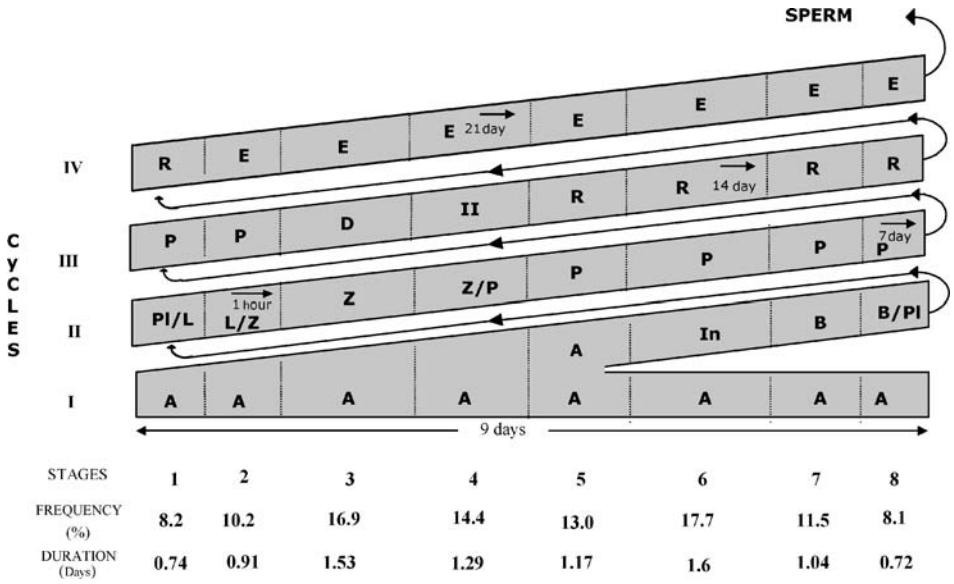

FIG 3. Diagram showing the germ cell composition, frequencies (percentage), and duration (in days) for each stage of the seminiferous epithelium cycle. Also depicted are the most advanced germ cell types labeled at the eight stages of the cycle at the different periods investigated (at 1 h and at 7, 14, and 21 days) following tritiated thymidine injections. Roman numerals indicate the spermatogenic cycle. The space given to each stage is proportional to its frequency and duration. The letters within each column indicate germ cell types present at each stage of the cycle. A indicates type A spermatogonia; In, intermediate spermatogonia; B, type B spermatogonia; Pl, preleptotene spermatocytes; L, leptotene; Z, zygotene; P, pachytene; D, diplotene; II, secondary spermatocytes; R, round spermatids; E, elongated spermatids.

observed at 14 days after thymidine injection (Fig. 2c), whereas at 21 days after thymidine injection, elongated spermatids at stage 4 were the most advanced germ cell types labeled (Fig. 2d).

Based on the most advanced germ cell types labeled at each period investigated after thymidine injections and on the knowledge of the stage frequencies, the mean ± SEM duration of the seminiferous epithelium cycle was estimated to be 9.1 ± 0.2 days (Table 2). The duration of the various stages of the cycle according to the tubular morphology system was determined, taking into account the cycle length and the percentage of occurrence of each stage (Fig. 3). The shortest duration (0.72 days) was in stage 8, whereas the longest duration (1.6 days) was in stage 6. Considering that approximately 4.5 cycles are necessary for the spermatogenic process to be completed, the total duration of spermatogenesis in wild boars was estimated to be 41 days. The duration of the spermatogonial phase of spermatogenesis was approximately 14 days, and the life spans of primary spermatocytes and spermatids were approximately 13 days and 14 days, respectively.

Testis Morphometry

The mean numbers of Leydig cells and SCs per testis were approximately 10 billion and 2.4 billion, respectively, and the mean numbers of Leydig cells and SCs per gram of testis were approximately 157 million and 42 million, respectively (Table 3). The estimated Leydig cell nuclear volume and cell size were 120 μm^3 and 400 μm^3, respectively. Significant and positive correlations ($P < 0.05$) were observed between the numbers of SCs per testis and the testis weight, the seminiferous tubule volume, and the lumen volume ($r = 0.85$, $r = 0.81$, and $r = 0.83$, respectively). A negative and significant correlation ($P < 0.05$) was found between the number of SCs per gram of testis and the tubular diameter ($r = -0.89$). The number of Leydig cells per testis showed significant correlations ($P < 0.05$) with the testis weight ($r = 0.79$) and with the number of SCs per testis ($r = 0.91$).

TABLE 3. Leydig and Sertoli cell morphometry and daily sperm production.

Parameter (n = 7)[a]	Mean ± SEM
Leydig cell nuclear diameter (μm)	6.0 ± 0.1
Leydig cell volume (μm^3)	399 ± 5
Nuclear volume (μm^3)	116 ± 11
Cytoplasm volume (μm^3)	283 ± 4
Leydig cell no. per gram of testis ($\times 10^6$)	157 ± 21
Leydig cell no. per testis ($\times 10^9$)	9.9 ± 2.7
Sertoli cell no. per gram of testis ($\times 10^6$)	42.4 ± 3.4
Sertoli cell no. per testis ($\times 10^9$)	2.4 ± 0.3
Daily sperm production per gram of testis ($\times 10^6$)	28.6 ± 1.5
Daily sperm production per testis ($\times 10^9$)	1.7 ± 0.3

[a] Number of animals utilized = n.

The SC efficiency in wild boars, estimated from the total number of round spermatids per SC, was approximately 7. The mean ± SEM meiotic index, measured as the number of round spermatids produced per pachytene spermatocyte, was 2.7 ± 0.2. These data show that almost one third of the germ cells were lost during the two meiotic divisions in the wild boar seminiferous epithelium. Although not statistically significant, there was almost a 15% loss of round spermatids during spermiogenesis in wild boars. The DSP values per testis and per gram of testis among wild boars in this study were 1.7 billion and 29 million, respectively (Table 3). The DSP showed significant correlations ($P < 0.05$) with the body weight ($r = 0.77$), the testis weight ($r = 0.97$), and the number of SCs per testis ($r = 0.87$).

DISCUSSION

To our knowledge, the present study is the first to report the results of a careful morphofunctional investigation of the testis structure and the spermatogenic process in the wild boar. Besides providing valuable data regarding the reproductive biology of this species, our results may allow important comparisons between the well-investigated domestic pig and the wild boar, which is phylogenetically the ancestor of the domestic pig.

Although wild boars in their natural European habitat show seasonal reproduction, as observed for ovarian and testis activity [27–30], reproduction in this species in the tropical climate of Brazil seems unaffected by season, as offspring are born throughout the year. For this reason, we did not investigate the influence of season on testis function.

The wild boars investigated in the present work were approximately 12 mo old, and for this reason the testis weight

TABLE 4. Comparative parameters related to the testis morphometry and spermatogenic events in sexually mature domestic and wild boars.

Parameter	Domestic boar[a]	Wild boar
Gonadosomatic index (%)	0.4	0.3
Seminiferous tubule (%)	83–85	87
Leydig cells (%)	7–19	6
Premeiotic stage frequency (%)	29–32	35
Postmeiotic stage frequency (%)	57–59	51
Spermatogenic cycle length (day)	8.6–9.0	9.0
Total duration of spermatogenesis (day)	38.7–40.5	40.5
Leydig cell individual size (μm^3)	1100–2200	400
Leydig cells per gram of testis (million)	60–120	157
Sertoli cells per gram of testis (million)	16–39	42
Sertoli cell efficiency (spermatids per SC)	12.4	6.6
Spermatogenic efficiency[b]	20–27	29

[a] These data are related to sexually mature boars and from several different pig breeds such as Yorkshire, Lacombe, Landrace, Whitecross, Piau, Meishan, and West African. Data were compiled from the literature (see reviews in França and Russell [12] and França et al. [17]), and at least six adult boars were utilized for each parameter considered.
[b] DSP per gram of testis.

values were probably approximately 20% higher than those found by Mauget and Boissin [30], whose animals were 2 mo younger. Despite this age difference, the GSIs obtained in both studies were similar. Compared with domestic pig breeds [31], the GSI was approximately 30% lower in wild boars. This may be explained by the extensive selection for reproductive performance to which the domestic pig breeds have been submitted. Table 4 compares several important testicular parameters between domestic pigs and wild boars.

One of the main causes of different spermatogenic efficiencies observed among mammalian species seems to be the variation in the proportion of tubular and intertubular compartments [12, 17, 32]. The mean percentage of the intertubular compartment in the wild boar was approximately two thirds of the reported value for the domestic pig [12, 31, 33]. This difference may be related to the low percentage of Leydig cells in the wild boar testis (6%) compared with that in domestic pigs (10%–15%) [12, 31, 33]. Because Leydig cells secrete steroids and pheromones that are important for other reproductive functions in boars (such as sexual behavior and maintenance of the male reproductive tract function and accessory glands), the domestic pig may have required a higher volume density of Leydig cells during the selective process for high reproductive efficiency. However, the number of Leydig cells per gram of testis observed for wild boars is considerably higher that the values cited for domestic pigs [12, 33] and is the highest found among mammalian species investigated to date [12, 34]. On the other hand, the individual Leydig cell size in wild boars was approximately 5-fold smaller than the mean size observed for domestic pigs [33] and represents one of the smallest Leydig cells observed among mammals [12, 34]. To our knowledge, there is no explanation in the literature about what determines the Leydig cell cytoarchitecture organization in the testis and its volume density and individual size.

Probably because of the phylogenetic proximity between wild boars and domestic pigs, the germ cell morphology and the different cellular associations characteristic of each stage of the seminiferous epithelium cycle were similar in these two swine subspecies [17, 35, 36]. However, the same was not true for the stage frequencies. For instance, stage 3, which presents the lowest frequency (~5%) in the domestic pig [35–38], was the second most frequent stage (~17%) in wild boars. Reports showing high variation of the stage frequencies among different individuals of the same mammalian species are not unusual in the literature [8, 9, 36, 39, 40, 41], and this feature was observed in wild boars investigated in the present study. However, when wild boars and domestic pigs are compared, the variation observed for the stage 3 frequency indicates that the pace of germ cells in this particular stage of spermatogenesis has changed drastically during evolution and domestication. The reason for this is unknown, but it is probably related to changes in the cell cycle or in the germ cell complement that is characteristic of this stage. Nevertheless, when the frequencies of different stages are grouped in premeiotic and postmeiotic phases of spermatogenesis, the values were similar for wild boars and domestic pigs [12, 35, 36, 42]. This is in agreement with several reports in the literature [12, 36, 40, 43, 44], suggesting that the phylogeny is strongly related to the frequencies of different stages when they are grouped in premeiotic and postmeiotic phases.

The autoradiographic technique we used allowed high-quality labeling for germ cells in the studies of the duration of the spermatogenic events. The values obtained in wild boars for the spermatogenic cycle length and the total duration of spermatogenesis were similar to those found for domestic pigs [35, 36]. Although high variation was observed among the frequencies for some stages and, consequently, for the germ cell pace, the total duration of spermatogenesis was not different in these two swine species. This finding is in general agreement with investigations showing that the spermatogenic cycle length is under the control of the germ cell genotype [15] and that the duration of spermatogenesis does not differ substantially among different breeds or strains [6, 12]. Each of the three phases of spermatogenesis (spermatogonial, meiotic, and spermiogenic) lasts approximately one third of the entire process [9, 12, 40], and the values obtained for these spermatogenic phases are similar in both swine species.

In mammals, only 2 or 3 of 10 expected spermatozoa are produced from differentiated type A spermatogonia, and the highest level of germ cell apoptosis occurs during the spermatogonial phase through a density-dependent regulation [45] and during meiosis due to chromosomal damage [12, 46]. However, similar to the findings in domestic pigs, in wild boars 10%–15% of germ cells are lost during spermiogenesis [17, 42]. The meiotic index observed for wild boars in the present study was similar to that found for other swine breeds [12] and is slightly higher than the values observed for most other species [32, 46], suggesting that this aspect of spermatogenesis is conserved in pigs.

Based on the fact that each SC supports a limited number of germ cells in a species-specific manner [47], the number of SCs established before puberty determines the rate of sperm production in sexually mature animals [16, 17, 48–50]. Taking these assumptions into consideration, the spermatogenic efficiency, expressed as the DSP per gram of testis, is usually positively correlated with the number of germ cells supported by each SC [12, 17, 32, 51]. The number of SCs per gram of testis for wild boars was much higher than that found in domestic swine breeds and in most other mammalian species [17, 38]. However, the SC efficiency in wild boars was almost 50% lower than that observed in domestic pig breeds. This result suggests that the reproductive selection process in domestic pigs has improved the SC efficiency, resulting in significantly higher testis weight and GSI compared with those in wild boars.

The spermatogenic efficiency is considered a useful parameter for interspecies comparisons among mammals [7, 12, 14, 17, 51]. The spermatogenic efficiency observed in wild boars is slightly higher than that found in domestic pigs [12, 17, 35, 38, 52–56]. As summarized in Table 4, this indicates

that the lower SC efficiency in wild boars is compensated by a higher number of SCs per gram of testis. However, because of the much smaller testis size observed in this species, its DSP per testis is low compared with that in domestic pigs [53].

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