2012

Spermatogenesis in goats with and without scrotum bipartition

Animal Reproduction Science, Amsterdam, v. 130, n.1-2, pp. 42-50, jan, 2012
http://www.producao.usp.br/handle/BDPI/32546

Animal Reproduction Science 130 (2012) 42–50

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Spermatogenesis in goats with and without scrotum bipartition

A.A.N. Machado Júnior[a,*], L.S. Oliveira[b], A.C. Assis Neto[c], F.R. Alves[a], M.A. Miglino[c], M.A.M. Carvalho[b]

[a] Universidade Federal do Piauí, Campus Profa Cinobelina Elvas, Curso de Medicina Veterinária, Bom Jesus, Piaui, Brazil
[b] Universidade Federal do Piauí, Centro de Ciências Agrárias, Departamento de Morfofisiologia, Teresina, Piaui, Brazil
[c] Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Clínica e Cirurgia Veterinária, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 1 December 2010
Received in revised form
20 December 2011
Accepted 21 December 2011
Available online 10 January 2012

Keywords:
Spermatogenesis
Germ cells
Bipartite scrotum
Goats
Sertoli cells

ABSTRACT

The objective of the present research was to quantify the seminiferous epithelium cells, spermatogenesis efficiency and characterize the ultrastrucure of Sertoli cells in goats. Eighteen goats were used and divided into three groups: Group I – goats without bipartition of the scrotum; Group II – animals with bipartition of the scrotum in up to 50% of the testicular length; Group III – goats with bipartition of the scrotum in more than 50% of the testicular length. The goat testes in Group III had a greater number of primary spermatocytes (25.37 ± 4.55 cells per cross sections), spermatids (112 ± 15.12 cells per cross sections), and Sertoli cells (9.46 ± 1.74 cells per cross sections) than the animals in Groups I and II ($P < 0.05$). The spermatogenic mitotic, meiotic, and general efficiency were greater in animals in Group III (1.25 ± 0.28; 5.12 ± 1.63; 6.44 ± 1.96) when compared to those in Groups I and II. Sheet-like processes originated from the Sertoli cell body as simple and smooth structures which involved almost all the surface of germ cells. Slender cord-like processes originated from Sertoli cells and also from the sheet-like processes. The relative frequency of the cycle stages showed differences among the groups of goats studied, and the highest frequency was in Stage 3 (20.68% for goats in Group I, 21.15% for those in Group II, and 16.89% for the animals in Group III). In conclusion, goats with bipartition of the scrotum have a greater number of germ and Sertoli cells per cross section of seminiferous tubule, that indicated a greater sperm production when compared to the other groups, and the ultrastructure of the Sertoli cell process did not present any relationship with bipartition of the scrotum.

1. Introduction

Cell quantification shows the normal standard of germ cell division and renewal, so that the spermatogenic efficiency coefficient can be determined (Castro et al., 1997). The spermatogenic efficiency, in practice, reflects on ratios found among different cell types that form the seminiferous epithelium in the tubule cross section, as described for ruminants, pigs, buffalo, monkeys, capybaras, and collared peccary and white-lipped peccaries (Cardoso and Godinho, 1985; Queiroz and Cardoso, 1989; Paula, 1999; Silva, 2000; Leal, 2004; Costa et al., 2004, 2007).

In the study of spermatogenesis, in addition to classifying the stages of the seminiferous epithelial cycle, studies should include quantification of the cell types in the seminiferous tubules, so evolution of these cells can be evaluated during the cycle (Almeida et al., 2000; Assis Neto et al., 2003a). According to Costa and Paula (2003), spermatogenic efficiency is not 100%, because there is

* Corresponding author at: Universidade Federal do Piauí, Campus Profa Cinobelina Elvas, BR 135, Km 3, Bairro Novo Horizonte, Bom Jesus, Piauí, CEP 64900-000, Brazil. Tel.: +55 89 3562 1866.
E-mail address: machadojunior2@yahoo.com.br (A.A.N. Machado Júnior).

0378-4320/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.anireprosci.2011.12.012

apoptosis of germ cells necessary to maintain intra-tubular homeostasis. Cell losses normally vary from 5% to 30% and are mainly observed during meiosis, so that about three round spermatides are formed from a primary spermatocyte (Sharpe, 1994; França and Russell, 1998).

Studies of spermatogenesis and the seminiferous epithelium cycle in goats was reported by Leal et al. (2004), but these aspects have never been associated with scrotal bipartition, a morphological variation found in goats raised in tropical regions. Studies with these animals have revealed some anatomical, physiological, and reproductive differences influenced by the presence of scrotal bipartition. Almeida et al. (2008) observed that in goats with bipartite scrotum the number of arterial branches in the region of bipartition was greater than that found in the region of scrotal raphe in goats without bipartition in the scrotum. Nunes (2005) and Nunes et al. (2010) identified that the number of sweat glands in the scrotal skin and the testicular artery length contained within the spermatic cord were higher in goats with scrotal bipartition. These aspects are directly related to the ability of the scrotum to lose heat to ensure a proper testicular thermoregulation to complete spermatogenesis (Machado Júnior et al., 2008). Recently Machado Júnior et al. (2011) reported that the scrotal bipartition is responsible for testicular morphological changes in the total length of the seminiferous tubule, total number of Sertoli and Leydig cells and daily sperm production in animals that have a bipartite scrotum.

The objective of the present research was to quantify the germ cells existing in the seminiferous epithelium, determine spermatogenic efficiency and seminiferous epithelium cycle stages and their respective relative frequencies, and comparatively analyze the Sertoli cell ultrastructure among goats with and without bipartition of the scrotum.

2. Materials and methods

The present study was conducted using 18 crossbred goats, aged between 1 and 1.5 years old, divided into three groups of six animals, according to the external configuration of the scrotum: Group I – goats without a bipartition of the scrotum; Group II – goats with a bipartition of the scrotum in up to 50% of the testicular length; Group III – animals with bipartition of the scrotum in more than 50% of the testicular length (The study was approved by the Research Ethics Committee – UFPI, no. 0540/06). The animals were raised in the northeast region of Brazil where the climate is tropical with rainfall mainly in the summer and autumn.

All the animals were weighed and submitted to orchiectomy under local anesthesia and both testes were obtained and weighed after their separation from epididymis. Testicular fragments approximately 3 mm tick, 5 mm wide, and 10 mm long were obtained from the proximal, middle, and distal end in relation to the abdominal wall, and placed in Bouin's solution under refrigeration (8 °C) for 24 h for histological processing and then embedded in paraffin (Assis Neto et al., 2003b).

Four μm thick sections were stained with Hematoxylin–Eosin and analyzed using a light microscope coupled to a Qwin D-1000V 4.1 computer system for image analysis (Leica microsystems – Aotec Instrumentos Científicos Ltda. Rua Afonso Celso, 1244, São Paulo 04119-061, Brazil). Different cell types (spermatogonia, pachytene primary spermatocytes, round spermatides, and Sertoli cells) were quantified from observation of 20 cross sections of seminiferous tubules with the roundest contour, in Stage 1 of the seminiferous epithelium, at 400× magnification. This count was corrected for nuclear (germ cells) or nucleolar (Sertoli cells) diameter and thickness of histological section, according to Abercrombie's formula (1946), modified by Amann and Almquist (1962):

$$\text{Corrected number} = \text{count obtained} \times \frac{\text{section thickness}}{\text{section thickness} + \sqrt{(\text{MD}/2)^2 - (\text{MD}/4)^2}}$$

To determine mean nuclear or nucleolar diameter (MD) 10 germ cell nuclei and 10 Sertoli cell nucleoli were measured in each section analyzed at 1000× magnification.

Spermatogenic efficiency was determined by obtaining the following ratios: mitosis efficiency or the mitosis efficiency coefficient: ratio between pachytene primary spermatocytes and spermatogonia in Stage 1; meiotic efficiency: ratio between round spermatides and pachytene primary spermatocytes in Stage 1; spermatogenesis general efficiency: ratio between round spermatides and primary spermatocytes in the pachytene in Stage 1; spermatogenesis general efficiency: ratio between round spermatides and spermatogonia in Stage 1; Sertoli cells efficiency: ratio between the total number of round spermatides in Stage 1 and the total number of Sertoli cells (Costa et al., 2007).

The Sertoli cells were classified into two types A and B according to the methodology proposed by Park et al. (1993). Type A Sertoli cells were associated with elongated spermatids and Type B Sertoli cells were associated with round and immature spermatids. The definition of cell processes took into account the morphology of these processes and the nomenclature used was that described by Kurohmaru and Nishida (1987) and Know et al. (2002).

The tubular morphology method that was used to characterize the stages of the seminiferous epithelium allowed for categorization through observing the modifications on the shape and position of the spermatide nucleus and the occurrence of meiotic division figures in the seminiferous epithelium that resulted in eight stages (Curtis, 1918; Ortavant et al., 1977). The relative frequency of the stages of the seminiferous epithelium cycle in goats was determined by analyzing, by means of horizontal scanning of 500 seminiferous tubule cross sections for each animal, at 400× magnitude.

All the results are presented as mean and standard deviation and they were obtained after analysis of variance for a completely randomized design using the Student–Newman–Keuls (SNK) test to compare mean values at the 5% level of significance.

3. Results

3.1. General results

Because there were no changes in testicular weight in the experimental group ($P > 0.05$): Group I (84.6 ± 27.8 g), Group II (85.5 ± 18.4 g), and Group III (89.4 ± 9.7 g), the differences among the groups of goats were thought to not be influenced directly by biometric features and rather by scrotal bipartition.

3.2. Quantitative analyses of spermatogenesis and Sertoli cell descriptions

Total numbers corrected of germ cells and Sertoli cells, by seminiferous tubule cross section, in Stage 1 of the seminiferous epithelium cycle in goats with different amounts of scrotal configurations are shown in Table 1. Spermatogonia did not show statistical differences among the three groups of goats studied.

Number of pachytene primary spermatocytes by cross section was different ($P < 0.05$) among groups. The animals in Group III had a greater number of these cells when compared with goats in Groups I and II.

Regarding the number of round spermatids found in Stage 1 of the cycle, there was a difference ($P < 0.05$) among the groups, similar to that described for the pachytene primary spermatocytes. Goats in Group III had a greater number of round spermatids per tubular cross section when compared with the goats in Groups I and II.

The number of Sertoli cells per tubular cross section in Stage 1 was different among the three groups ($P < 0.05$). The animals in Group I had fewer cells, when compared with Groups II and III ($P < 0.05$), demonstrating that goats with a greater amount of scrotal bipartition had a greater number of Sertoli cells.

Spermatogenic efficiency (Table 2) was obtained based on the ratio among different cells that form Stage 1 of the seminiferous epithelium cycle (Table 1). This efficiency was divided into mitotic efficiency, meiotic efficiency, and general spermatogenesis efficiency. Mitotic efficiency or the mitotic efficiency coefficient of animals in Group III was different ($P < 0.05$) from that found for goats in Groups I and II.

The calculated values for meiotic and mitotic efficiency and the general spermatogenesis efficiency indicated differences when Groups I and II were compared to Group III; there was, however, no difference for these values between Groups I and II.

The ratio among the number of round spermatids supported by a Sertoli cell provided the efficiency or Sertoli cell index (Table 2). In this aspect, it can be observed that goats in groups with bipartition of the scrotum (Groups II and III) had greater values ($P < 0.05$) than the group of animals that did not have this morphological characteristic (Group I).

No description was found in the literature about the ultrastructure of Sertoli cell processes in goats raised in Brazil. It was observed that the cell processes of both Types A and B Sertoli cells had sheet-like and slender cord-like processes. The sheet-like processes began in the Sertoli cell body as simple and smooth structures which covered almost all the surface of the germ cells. Processes involving spermatids were observed in the medial and apical portions of the seminiferous epithelium and had a flatter aspect (Fig. 1A).

Slender cord-like processes were observed in different areas. Processes found in the medial and apical portion of seminiferous tubules projected both from the Sertoli cell body and the sheet-like processes and were attached to round spermatids acquiring an elliptical shape (Fig. 1B).

3.3. Morphological characterization and frequency of the seminiferous epithelium cycle stages

The stages of the seminiferous epithelium cycle had similar cellular associations, regardless of group, either with or without bipartite scrotum:

3.3.1. Stage 1

Round spermatides, which also have round nuclei, were arranged in several layers inside the seminiferous epithelium. Sertoli cells had in the nucleus an obvious nucleolo. Spermatogonia were located close to the basal lamina. The only generation of primary spermatocytes was in the pachytene phase and was located between the round spermatids, spermatogonia and Sertoli cells nucleus (Fig. 2A).

3.3.2. Stage 2

Oval-shaped spermatids, starting the enlargement process of their cytoplasm and with heads pointed toward the Sertoli cell nucleus, were located at the base of the seminiferous tubule. Primary spermatocytes in preleptotene/leptotene were observed very close to the basal lamina and the spermatocytes in pachytene were distributed under these first cells toward the tubular lumen. The nuclei and nucleolus of Sertoli cells and the spermatogonia were observed, as described in the previous stage (Fig. 2B).

3.3.3. Stage 3

Spermatides, at a more advanced stage of enlargement, started to form the first bundles of cells with elongated nuclei. There were two populations of primary spermatocytes, in zygotene and diplotene, with large and relatively spherical nuclei. Sertoli cell and spermatogonia were verified close to the basal lamina of the seminiferous tubules (Fig. 2C).

3.3.4. Stage 4

Elongated spermatid bundles became more evident. A striking feature of this stage was the occurrence of two meiotic divisions, spermatocytes in diplotene developing into secondary spermatocytes that then modified into round spermatides. Spermatogonia and primary spermatocytes in zygotene were very evident at this stage. The Sertoli cells had similar morphology to that observed in the previous stages. Meiotic division figures were also present at this stage (Fig. 2D).

3.3.5. Stage 5

Round spermatids and elongated spermatids grouped into cellular bundles. The round spermatid morphology

Table 1
Corrected number of germ cells and Sertoli cells per cross section of seminiferous tubules in stage 1 in goats with and without scrotal bipartition.

Group	A	P	R	S
I ($n=6$)	18.10 ± 5.62^{a}	20.01 ± 6.6^{b}	89.20 ± 22.40^{b}	7.86 ± 1.9^{c}
II ($n=6$)	19.36 ± 5.07^{a}	21.64 ± 4.67^{b}	96.71 ± 19.82^{b}	8.67 ± 2.2^{b}
III ($n=6$)	20.18 ± 6.06^{a}	25.37 ± 4.55^{a}	130 ± 15.12^{a}	9.46 ± 1.74^{a}

Mean values within column with different superscript letters differ ($P<0.05$). n = number of goats per treatment.
A, spermatogonium; P, Pachytene primary spermatocyte; R, round spermatide; S, Sertoli cells.

Table 2
Spermatogenesis efficiency and Sertoli cell efficiency in goats with and without bipartition of the scrotum.

Group	ME	MeE	GES	SCE
I ($n=6$)	1.1 ± 0.27^{b}	4.45 ± 1.08^{b}	4.92 ± 1.5^{b}	10.57 ± 4.61^{b}
II ($n=6$)	1.11 ± 0.28^{b}	4.46 ± 0.82^{b}	4.99 ± 1.47^{b}	12.72 ± 3.97^{a}
III ($n=6$)	1.25 ± 0.28^{a}	5.12 ± 1.63^{a}	6.44 ± 1.96^{a}	13.64 ± 2.64^{a}

Mean values within column with different superscript letters differ ($P<0.05$). n = number of goats per treatment.
ME, mitotic efficiency; MeE, meiotic efficiency; GES, general spermatogenesis efficiency; SCE, Sertoli cells efficiency.

was similar to the secondary spermatocytes of Stage 4, and had a smaller cellular diameter than that of the spermatocytes. Elongated spermatid bundles were within crypts of Sertoli cells forming two [well evident] very clear columns. Primary spermatocytes in zygotene and pachytene were found interspersed between round spermatids and basal lamina (Fig. 3A).

3.3.6. Stage 6

There were elongated spermatid bundles facing toward the tubular lumen and starting the dissociation process. Several layers of round spermatids were observed. The primary spermatocytes in pachytene were similar to those observed at Stage 5. The Sertoli cells expressed more developed nuclei and most had the long axis perpendicular to the basal lamina of the seminiferous tubules (Fig. 3B).

3.3.7. Stage 7

Elongated spermatids were located close to the tubular lumen and almost completely dissociated, and cellular bundles were prevalent differing from Stages 3 to 6 in this regard. Primary spermatocytes in pachytene had similar nuclei morphology to those observed in the previous stage. Other cells observed were spermatogonia, Sertoli cells, and round spermatides (Fig. 3C).

3.3.8. Stage 8

Elongated spermatides were completely differentiated into spermatozoa and in the release process from Sertoli cells. The residual bodies resulting from spermatozoa formation process were more frequently observed. Spermatogonia, Sertoli cells, primary spermatocytes in pachytene, and round spermatides were also observed at this stage (Fig. 3D).

3.4. Frequencies of stages of the seminiferous epithelium

Table 3 provides the relative frequency of the stages in the seminiferous epithelium, calculated after determining the cell association that characterized the stages of the seminiferous epithelium cycle. Stage 3 of the cycle had the greatest frequencies (20.68% in Group I; 21.15% in Group II; and 16.89% in Group III) ($P>0.05$); the least relative frequency was verified for Stage 2, in Groups I (8.53%) and II (7.20%) ($P>0.05$), while in animals in Group III the stage that was least frequent was 8 (8.37%) ($P<0.05$).

The frequencies of the pre-meiotic (Stages 1–3), meiotic (Stage 4), and post-meiotic (Stages 5–8) phases of the seminiferous epithelium cycle were 42.29, 13.80, and 43.86% in Group I; 38.51, 11.15, and 50.14% in Group II; and 40.04, 12.90, and 47.03% in Group III, respectively.

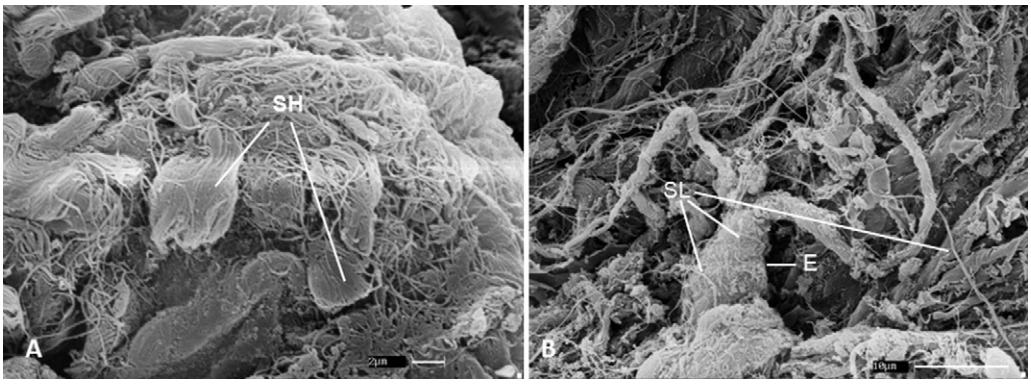

Fig. 1. Scanning electromicrographic of the Sertoli cells, showing the sheet-like processes (SH) as simple and smooth structures covering the germ cells. Bar = 2 μm (A) and showing the slender cord-like processes (SL), involving an elongated spermatide (E). Bar = 10 μm (B).

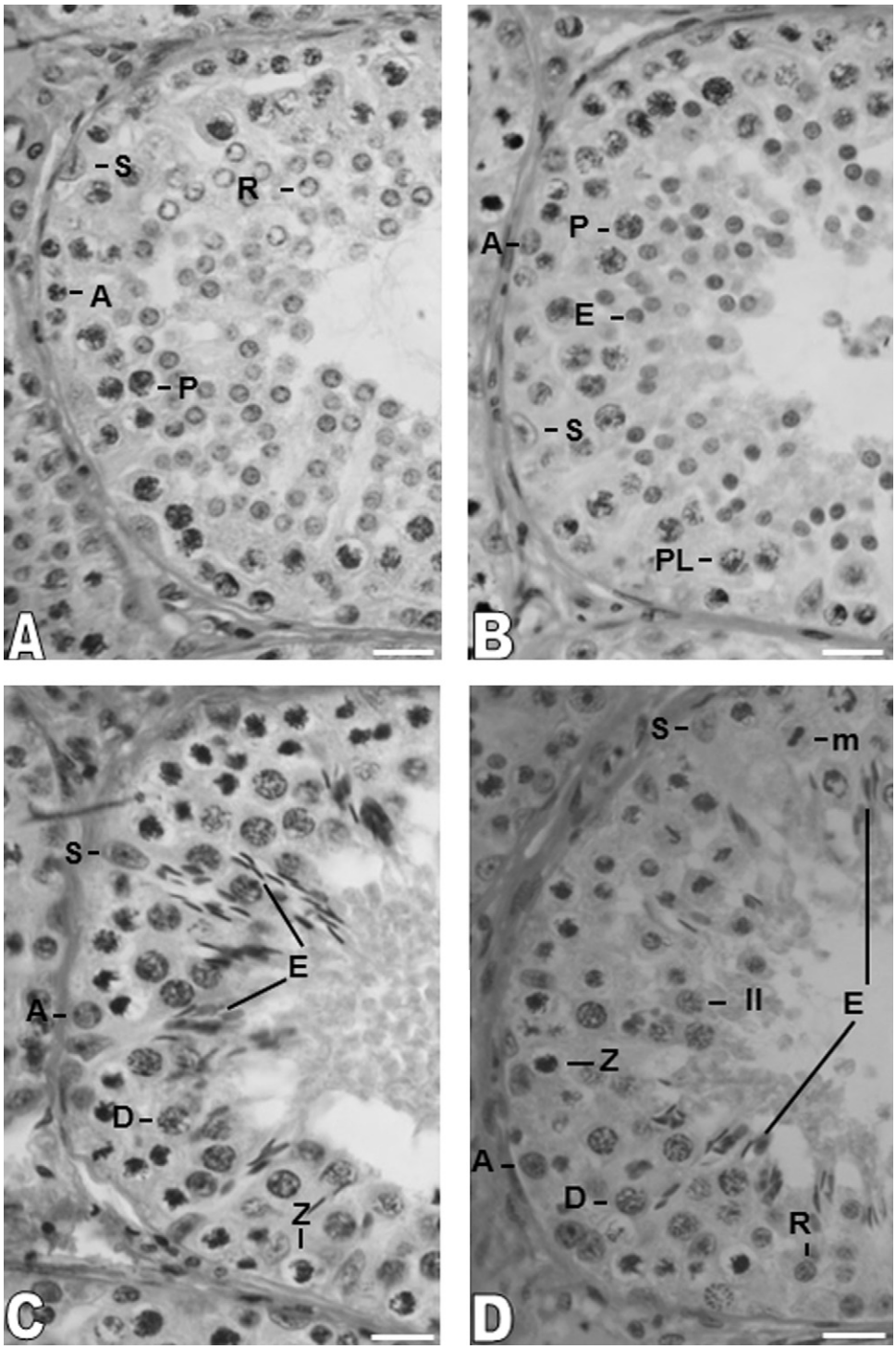

Fig. 2. Photomicrography of the testis of goats in Group I, in cross sections, showing the Stages 1 (A), 2 (B), 3 (C), and 4 (D) of the seminiferous epithelium cycle with spermatogonia (A), primary spermatocytes in preleptotene/leptotene (PL), zygotene (Z), pachytene (P), and diplotene (D), secondary spermatocytes (II), round spermatides (R), and elongated spermatides (E), Sertoli cell (S) and meiotic figures (m), in cell associations; bar = 50 μm.

4. Discussion

Previous findings (Castro et al., 1997; França and Russell, 1998; Leal, 2004) indicate that populations of germ and Sertoli cells increase during animal development and, from puberty and then tend to stabilize. Thus the quantity of these cells existing in the seminiferous epithelium can be used to estimate the reproductive capacity of an animal, as

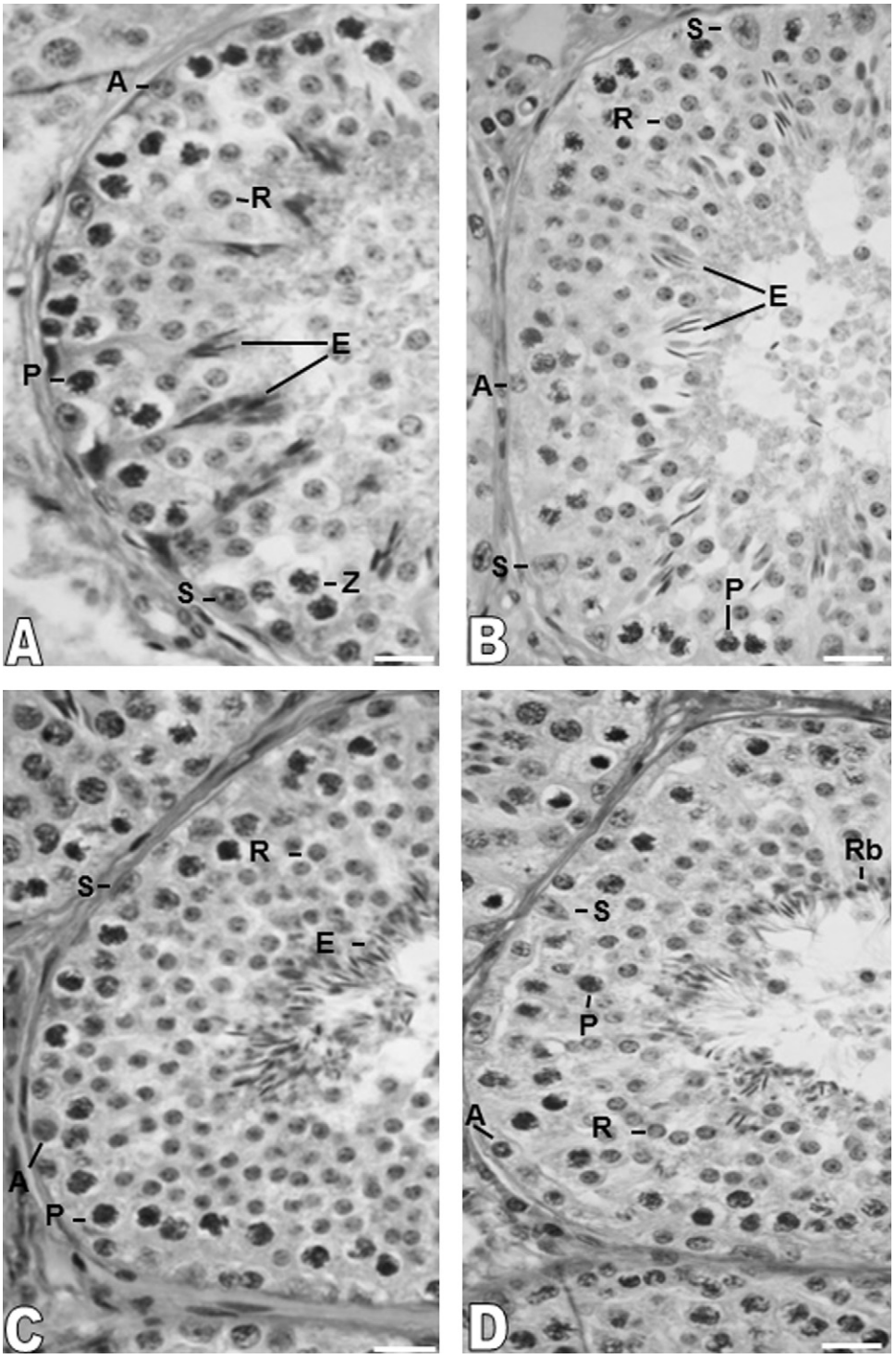

Fig. 3. Photomicrography of the testis of goats in Group III, in cross sections, showing the Stages 5 (A), 6 (B), 7 (C), and 8 (D) with spermatogonia (A), primary spermatocytes in zygotene (Z), pachytene (P), round spermatides (R) and elongated spermatides (E), Sertoli cell (S) and residual bodies (Rb), in cells associations; bar = 50 μm.

well as to identify the quantity and organization of these cells in the testes, so that comparative evaluations can be made of animals exposed to experimental and pathological situations.

The spermatogonia number, although it did not vary among the groups of goats in the present study, was greater than that reported previously in several species (França et al., 1988; Silva, 1996; Paula, 1999; Assis Neto et al.,

Table 3
Mean ± standard deviation of relative frequency (%) of the stages of the semineferous epithelium cycle in goats with and without bipartite scrotum.

	Group I	Group II	Group III
Stage 1	13.08 ± 1.1[a]	10.16 ± 0.9[b]	13.02 ± 1.2[a]
Stage 2	8.53 ± 0.8[ab]	7.20 ± 1.1[b]	10.13 ± 2.1[a]
Stage 3	20.68 ± 1.3[a]	21.15 ± 1.5[a]	16.89 ± 0.8[b]
Stage 4	13.80 ± 0.7[a]	11.15 ± 0.5[b]	12.90 ± 1.1[a]
Stage 5	10.43 ± 1.2[a]	11.84 ± 1.0[a]	13.75 ± 2.1[a]
Stage 6	11.51 ± 0.7[b]	16.48 ± 1.3[a]	15.76 ± 0.9[a]
Stage 7	9.18 ± 0.6[a]	9.49 ± 0.4[a]	9.15 ± 0.7[a]
Stage 8	12.74 ± 1.1[a]	12.33 ± 1.3[a]	8.37 ± 0.9[b]

Mean values followed by different letters are different ($P < 0.05$) among the groups.

2003a; Costa et al., 2004, 2007; Leal, 2004). The number of pachytene primary spermatocytes was greater in the goats with greater bipartition of the scrotum (Group III) when compared with the animals in Groups I and II. This number was similar to that reported for Saanen goats (Silva, 2000) and differed from that of hairless sheep (Queiroz and Cardoso, 1989) without scrotal bipartition.

In Stage 1 of the seminiferous epithelium cycle in goats, only one generation of primary spermatocyte results in the quantity of these cells in this species being different from that observed in other animals such as capybaras (Paula, 1999), agoutis (Assis Neto et al., 2003a), marmosets (Leal, 2004), collared peccaries (Costa et al., 2004), white-lipped peccaries (Costa et al., 2007), in which two generations of primary spermatocytes were detected. In some animals such as the gerbil (Segatelli et al., 2004) which have only one generation of primary spermatocytes in Stage 1, the number of these cells resembles that found for goats with different scrotal conformations.

The number of round spermatids per cross section of seminiferous tubule observed in the goats in the present research was greater than that observed previously for agoutis, pigs, monkeys, white-lipped peccaries, collared peccaries, capybaras, and goats (França et al., 1988; Silva, 1996, 2000; Paula, 1999; Assis Neto et al., 2003a; Costa et al., 2004, 2007; Leal, 2004). This number was also different among the groups of goats in the present research.

Thus, the animals in Group III of the present study had a number of spermatids and primary spermatocytes that were greater than the goats in Groups I and II. This finding showed that the animals with greater scrotal division tended to have a more germ cells per cross section when compared to those which did not present this characteristic or even those where the bipartition of the scrotum was less evident. These quantitative differences may occur due to a differentiated spermatogenic process in these animals, where cell loss (apoptosis) may be less in goats with greater bipartition of the scrotum than in the group of animals with partial bipartition of the scrotum and those without bipartition of the scrotum. Although the germ cell is regulated by apoptotic mechanisms, according to the number of Sertoli cells, the group of goats with bipartite scrotum had the greatest number of Sertoli cells in the testis (Machado Júnior et al., 2011), leading to the supposition that the apoptotic rate was less in these animals.

Another important factor that may have contributed to this difference of germ cells among the groups of goats in the present study was the number of Sertoli cells per tubular cross section. According to Rocha et al. (1999), the number of these cells per testis is the main factor to determine spermatic production and testicular size. This information is based on the fact that Sertoli cells have a relatively fixed support capacity for germ cells, so, the more Sertoli cells existing in an animal testis, the greater the number of germ cells there will be in a testis (Orth et al., 1988; França and Russell, 1998).

The goats in Group III had a greater number of Sertoli cells than the animals in Groups I and II, however, this was similar to the number found in several other animals (França et al., 1988; Silva, 1996; Paula, 1999; Assis Neto et al., 2003a; Costa et al., 2004, 2007; Leal, 2004).

Regarding the spermatogenic efficiency and Sertoli cell index, it was observed that the animals in Group III had greater values when compared to the animals in the other groups. Mitotic efficiency was less when compared to that observed by Silva (2000) in Saanen goats, Costa et al. (2004) in collared peccaries, Leal (2004) in marmosets, Costa et al. (2007) in white-lipped peccaries, and similar to that reported by Assis Neto et al. (2003a) in agoutis. These differences and similarities resulted because of the greater number of spermatogonia found in goats with different scrotal conformations.

It is hypothesized that the influence of the scrotal bipartition on the spermatogenic efficiency is mainly due to a greater number of Sertoli cells in the testes of goats with greater bipartition, as reported by Machado Júnior et al. (2011). The role of the number of Sertoli cells on the spermatogenic process is known, given its ability to create an intratubular environment to support germ cells differentiation. Thus, a greater number of these cells in the testis provide enhanced support for the development of germ cells, providing for more efficient spermatogenesis.

Meiotic efficiency, however, was greater than that observed for white-lipped peccaries, collared peccaries, and capybaras (Paula, 1999; Costa et al., 2004, 2007), and similar to that for marmosets and Saanen goats (Silva, 2000; Leal, 2004) and less than that reported by Assis Neto et al. (2003a) for agoutis.

The Sertoli cell efficiency index in the goats researched was similar to that for goats, marmosets, collared peccaries, and white-lipped peccaries (Silva, 2000; Leal, 2004; Costa et al., 2004, 2007) and it was less than that found by Silva (1996) for pigs, Paula (1999) for capybaras, and Assis Neto et al. (2003a) for agoutis.

The sheet-like and slender cord-like processes did not vary according to scrotal conformation and were similar for goats of all groups in the present study. Similar results were reported by Know et al. (2002) for Korean native goats, but different shapes were observed for the slender cord-like processes in these goats that were characterized according to the relationship with germ cells. However, these different shapes were more evident before the spermiation process when the residual body was detaching from the spermatid head (Park et al., 1993).

Hamasaki and Murakami (1986) related the occurrence of four types of processes in rat Sertoli cells: bough-like

process, sheet- or sleeve-like process, retracted process, and wrapper-like process. In Shiba goats the Sertoli cell processes had a slender, ramifying, and ring-like shaped aspect (Kurohmaru and Nishida, 1987). The shape of Sertoli cell processes varied according to Seminiferous tubule portion and evolution of the spermatogenic process.

For both Shiba goats (Kurohmaru and Nishida, 1987) and Korean native goats (Know et al., 2002) the sheet-like process contour was simple and smooth similar to that observed for the goats with different scrotal conformations used in the present study. But for the Syrian hamster (Gravis, 1975), monkey (Russell et al., 1986), and rat (Hamasaki and Murakami, 1986) the contour of theses processes was irregular and more complex than that found in the goats in the present study.

There is no previous report regarding the stages of the semineferous epithelium cycle in goats, considering different scortal conformations. Research such as that by França et al. (1999) and Onyango et al. (2000) described these stages in goats and found differences related to organization of cells.

The stages observed in the present research were similar to those observed by França et al. (1999), the only difference was related to the spermatogonia generations. This fact might have occurred due to the staining adopted in the present study that did not permit a suitable visualization of A, B, and intermediary type spermatogonia. Other cells reported by França et al. (1999) were also observed in the goats of the present study.

As shown by Onyango et al. (2000), Stages 5, 6, and 7 were described as a single stage. Stages 6 and 7 were considered an extension of Stage 5 because of the short duration of the phase. In the present research, these stages were described separately because the cell associations in each stage and the disposition in the seminiferous epithelium were different.

There are two classical methods for determining the stages of the seminiferous epithelium cycle. One method is based on the change that occurs in the shape and location of the spermatid nuclei and in the presence of meiotic divisions, forming 8 stages (Curtis, 1918; Ortavant et al., 1977) and the other method considers as parameter the spermatid acrosomal development, forming up to 14 stages (Leblond and Clermont, 1952). Both methods are efficient for demonstrating the cyclic event of spermatogenesis, and the acrosomal development method is used more for rodents (Leblond and Clermont, 1952; Russell et al., 1990; Onyango et al., 2000).

The method used for the present research identified 8 stages and permitted comparison with other domestic animals, such as cattle, sheep, and goats (Hochereau-de-Reviers et al., 1964; França et al., 1999; Onyango et al., 2000; Horn et al., 2003), pigs (Swierstra, 1968; França and Cardoso, 1998; Almeida et al., 2006), where the stages of the seminiferous epithelium had already been identified.

The results obtained for the frequency of the stages of the seminiferous epithelium cycle in goats with different scrotum conformations were similar to those reported by França et al. (1999) who referred to Stage 3 as the most frequent stage of the seminiferous epithelium cycle. However, the stage which occurs the least frequently, França et al. (1999) is Stage 7, while in the present research, Stage 2 was less frequent, among the goats in Groups I and II, and Stage 8 least frequent in Group III.

Onyango et al. (2000) described Stage 1 as being the most frequent, differing from that observed in the present research. However, there is a point where the results obtained by Onyango et al. (2000) are similar to those found in the present study, that is, Stage 8 was less frequent in the cycle which was verified in Group III in the present study.

In conclusion, the goats that had bipartition of the scrotum had a greater number of germ and Sertoli cells per section of seminiferous tubule, therefore, greater spermatogenic efficiency occurred when compared with the goats without bipartition of the scrotum. Sertoli cell processes in goats, sheet like and slender cord-like, did not have any relationship with bipartition of the scrotum. The cell associations that composed the stages of the seminiferous epithelium cycle in goats did not vary according to the scrotum conformation; however, the relative frequency of these stages varied among the different groups of goats, and this may reflect in the daily spermatic production of the animals.

Acknowledgements

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, processo: 620240/2006-7.

References

Abercrombie, M., 1946. Estimation of nuclear population from microtome sections. Anat. Rec. 92, 239–247.

Almeida, L.M., Weiss, R.R., Castro, C.S., Büchele, J., 2000. Quantificação histológica da espermatogênese em ratos Wistar tratados com dimetil sulfóxido. Arch. Vet. Sci. 5, 129–135.

Almeida, F.F.L., Leal, M.C., França, L.R., 2006. Testes morphometry, duration of spermatogenesis and spermatogenic efficiency in wild boar (*Sus scrofa scrofa*). Biol. Reprod. 75, 792–799.

Amann, R.P.A., Almquist, J.O., 1962. Reproductive capacity of dairy bull. VIII. Direct and indirect measurement of testicular sperm production. J. Dairy Sci. 45, 774–781.

Almeida, M.M., Carvalho, M.A.M., Machado Júnior, A.A.N., Righi, D.A., Xavier, F.G., Conde Júnior, A.M., Bombonato, P.P., 2008. Efeito do grau de bipartição escrotal sobre a vascularização arterial do escroto de caprinos nativos do Estado do Piauí. Braz. J. Vet. Res. Anim. Sci. 45 (3), 167–173.

Assis Neto, A.C., Melo, M.I.V., Carvalho, M.A.M., Miglino, M.A., Oliveira, M.F., Ambrósio, C.E., Silva, S.M.M.S., Blasquez, F.X.H., Papa, P.C., Kfoury Júnior, J.R., 2003a. Quantificação de células dos tubules seminíferos e rendimento da espermatogênese em cutias (*Dasyprocta aguti*) criadas em cativeiro. Braz. J. Vet. Res. Anim. Sci. 40, 175–179.

Assis Neto, A.C., Melo, M.I.V., Carvalho, M.A.M., Miglino, M.A., Oliveira, M.F., Menezes, D.J.A., Papa, P.C., Kfoury Júnior, J.R., 2003b. Análise qualitativa do estabelecimento da espermatogênese em cutias (*Dasyprocta aguti*) criadas em cativeiro. Braz. J. Vet. Res. Anim. Sci. 40, 180–184.

Cardoso, F.M., Godinho, H.P., 1985. Daily sperm production of zebus (*Bos indicus*) estimated by quantitative histology of the testis. Theriogenology 23, 841–849.

Castro, A.C.S., Bernedson, W.E., Cardoso, F.M., 1997. Cinética e quantificação da espermatogênese: bases morfológicas e suas aplicações em estudos da reprodução de mamíferos. Rev. Bras. Reprod. Anim. 21, 25–34.

Costa, D.S., Menezes, C.M.C., Paula, T.A.R., 2007. Spermatogenesis in white-lipped peccaries (*Tayassu pecari*). Anim. Reprod. Sci. 98, 322–334.

Costa, D.S., Henry, M., Paula, T.A.R., 2004. Espermatogênese de cateto (*Tayassu tajacu*). Arq. Bras. Med. Vet. Zootec. 56, 46–51.

Curtis, G.M., 1918. The morphology of the mammalian seminiferous tubule. Am. J. Anat. 24, 339–394.

Costa, D.S., Paula, T.A.R., 2003. Espermatogênese em mamíferos. Scientia 4, 53–72.

França, L.R., Cardoso, F.M., Castro, A.C.S., 1988. Desenvolvimento testicular de suíno Piau. IV. População celular dos túbulos seminíferos e rendimento da espermatogênese. Arq. Bras. Med. Vet. Zootec. 40, 339–354.
França, L.R., Cardoso, F.M., 1998. Duration of spermatogenesis and sperm transit time through the epididymis in the piau boar. Tissue Cell 30, 573–582.
França, L.R., Russell, L.D., 1998. The testis of domestic animals. In: Regadera, J., Martinez-Garcia (Eds.), Male Reproduction. Churchill Livingstone, Madrid, pp. 197–219.
França, L.R., Becker-Silva, S.C., Chiarini-Garcia, H., 1999. The length of the cycle of seminiferous epithelium in goats (*Capra hircus*). Tissue Cell 31, 274–280.
Gravis, C.J., 1975. Scanning electron microscopic study of the Sertoli cell and spermiation in the Syrian hamster. Am. J. Anat. 151, 21–38.
Hamasaki, M., Murakami, M., 1986. Three-dimensional profiles of Sertoli cells processes and the associated appendices as viewed by SEM. J. Electron Microsc. 35, 132–143.
Hochereau-de-Reviers, M.T., Courot, M., Ortavant, R., 1964. Marquage des cellules germinales du bélier et du taureau par injection de thymidine tritiée dans l'artère spermatique. Ann. Biol. Anim. Biochim. Biophys. 2, 157–161.
Horn, M.M., Moraes, J.C.F., Edelweiss, M.I.A., 2003. Quantificação dos estádios do ciclo espermatogênico em touros de raças sintéticas com e sem alteração na qualidade seminal. Ciência Rural 33, 1111–1115.
Know, G.O., Park, Y.S., Lee, S.H., Yamamoto, E., Hosaka, Y., Ueda, H., Takehana, K., 2002. Ultrastructure and function of Sertoli cell processes in the Korean Native Goat. Okaj. Fol. Anat. Jpn. 79, 121–128.
Kurohmaru, M., Nishida, T., 1987. Three-dimensional structure of the Sertoli cell in the Shiba goat. Arch. Histol. Jpn. 50, 515–523.
Leblond, C.P., Clermont, Y., 1952. Spermiogenesis of rat, mouse, hamster and guinea pigs as revealed by the periodic acidfuchsin sulfurous acid technique. Am. J. Anat. 90, 167–214.
Leal, M.C., Becker Silva, S.C., Chiarini Garcia, H., França, L.R., 2004. Sertoli cell efficiency and daily sperm production in goats (*Capra hircus*). Anim. Reprod. 1, 122–128.
Leal, M.C., 2004. Análise morfométrica e funcional do testículo e eficiência espermatogênica em Sagüis Callithrix penicillata (Primates: Callitrichidae). Belo Horizonte, 88p. Dissertação (Mestrado em Ciências), Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.
Machado Júnior, A.A.N., Oliveira, L.S., Conde Júnior, A.M., Menezes, D.J.A., Assis Neto, A.C., Alves, F.R., Carvalho, M.A.M., 2008. Cellular characterization and quantification of seminiferous ephitelium in goats with and without bipatition of the scrotum. Braz. J. Morphol. Sci. 25 (1–4), 45–45.
Machado Júnior, A.A.N., Assis Neto, A.C., Sousa Júnior, A., Menezes, D.J.A., Alves, F.R., Sousa, A.L., Carvalho, M.A.M., 2011. Daily sperm production and testicular morphometry in goats according to external scrotal conformation. Anim. Reprod. Sci. 127, 73–77.
Nunes, A.S., 2005. Morfologia do funículo espermático e dos escrotos em caprinos nativos do Estado do Piauí, com diferentes configurações escrotais. Teresina, 57p. Dissertação (Mestrado em Ciência Animal) – Universidade Federal do Piauí.
Nunes, A.S., Cavalcante Filho, M.F., Machado Júnior, A.A.N., Abreu Silva, A.L., Conde Júnior, A.M., Souza, J.A.T., Carvalho, M.A.M., 2010. Descrição histológica do escroto de caprinos nativos do Estado do Piauí, segundo o grau de bipartição escrotal. Ciência Rural 40 (8), 1808–1813.
Onyango, D.W., Wango, E.O., OtiangïA-Owiti, G.E., Oduor-Okelo, D., Werner, G., 2000. Morphological characterization of the seminiferous cycle in the goat (*Capra hircus*): a histological and ultrastructural study. Anat. Anz. 182, 235–241.
Orth, J.M., Gunsalus, G.L., Lamperti, A.A., 1988. Evidence from Sertoli cell depleted rats indicates that spermatid in adults depends on numbers of Sertoli cells produced during perinatal development. Endocrinology 122, 787–794.
Ortavant, R., Courot, M., Hochereau-de-Reviers, M.T., 1977. Spermatogenesis in domestic mammals. In: Cole, H.H., Cupps, P.T. (Eds.), Reproduction in Domestic Mammals. , 3rd ed. Academic Press, New York, pp. 203–227.
Paula, T.A.R., 1999. Análise histométrica e funcional do testículo de capivaras (*Hidrochoerus hydrochaeris*) adultas. Belo Horizonte, 84p. Tese (Doutorado em Biologia Celular) – Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.
Park, Y.S., Abe, M., Takehana, K., Iwasa, K., 1993. Three-dimensional structure of dog Sertoli cells: a computer aided reconstruction from serial semi-thin sections. Arch. Histol. Cytol. 56, 65–73.
Queiroz, G.G., Cardoso, F.M., 1989. Avaliação histológica do rendimento da espermatogênese de carneiros deslanados adultos. Rev. Bras. Reprod. Anim. 13, 99–108.
Rocha, D.C.M., Debeljuk, L., França, L.R., 1999. Exposure to constant light during testis development increase daily sperm production in adult Wistar rats. Tissue Cell 31, 372–379.
Russell, L.D., Ettlin, R.A., Sinha-Hikin, A.P., Clegg, E.D., 1990. Histological and Histopathological Evaluation of the Testis. Cache River Press, Clearwater, Florida, 286p.
Russell, L.D., Gardner, R.T., Weber, J.E., 1986. Reconstruction of a type B configuration monkey Sertoli cell: size, shape and configurational and specialized cell-to-cell relationship. Am. J. Anat. 175, 73–90.
Segatelli, T.M., França, L.R., Pinheiro, P.F.F., Almeida, C.C.D., Martinez, M., Matinez, F.E., 2004. Spermatogenic cycle length and spermatogenic efficiency in the Gerbil (*Meriones unguiculatus*). J. Androl. 25, 872–880.
Sharpe, R.M., 1994. Regulation of spermatogenesis. In: Knobil, E., Neil, J.D. (Eds.), The Physiology of Reproduction. , 2nd ed. Raven Press, New York, pp. 1363–1434.
Silva, S.C.B., 2000. Caracterização histológica e seminal do desenvolvimento sexual de caprinos saanem, criados em sistema intensivo. Belo Horizonte, 117p. Dissertação (Mestrado em Reprodução Animal) – Universidade Federal de Minas Gerais.
Silva, S.M.M.S., 1996. Desenvolvimento testicular de suínos das raças Large White e Landrace com diferentes taxas de ganho de peso diário. Belo Horizonte, 106p. Dissertação (Mestrado em Patologia Animal) – Universidade Federal de Minas Gerais.
Swierstra, E.E., 1968. Cytology and duration of the cycle of seminiferous epithelium of the boar; duration of spermatozoan transit through the epididymis. Anat. Rec. 161, 171–186.