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Sertoli cell efficiency and daily sperm production in goats (*Capra hircus*)

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Abstract

Although the goat is an economically important domestic mammal, there are very few data available in the literature for male reproductive biology in this species, mainly data related to testis morphometry obtained with high-quality light microscopy. Work recently developed in our laboratory found that the duration of the seminiferous epithelium cycle in goats is 10.6 days (França et al., 1999), and this finding allowed, for the first time, to estimate precisely the daily sperm production per gram of testis (spermatogenic efficiency) in this species and to compare this parameter with other mammalian species. Four sexually-mature Alpine bucks (*Capra hircus*; 25.3 ± 1.0 months of age and 59.3 ± 5.4kg of body weight) were utilized composing an homogeneous group.The mean testis weight in goats was 101g and the values found for seminiferous tubule and Leydig cell volume density (%) in goats were ~88% and ~1.5%, respectively. The mean seminiferous tubule diameter was 237µm, whereas the tubular length per testis and per testis gram was 1,829 meters and 20 meters, respectively. The number of round spermatids per pachytene primary spermatocytes (meiotic index) was 2.8, showing that, from the theoretical number of spermatids expected, 30% of cell loss occurred during the two meiotic divisions. The total number of germ cells and the number of round spermatids per Sertoli cell nucleolus (Sertoli cell efficiency) at stage 1 of the cycle were 24 and 15.4, respectively. This Sertoli cell support capacity is the highest among the domestic mammals species investigated. The Leydig cell volume was approximately 780µm^3 and the nucleus volume 170µm^3. Both Leydig and Sertoli cells number per gram of testis were ~20 million, and the daily sperm production per gram of testis in goats (efficiency of spermatogenesis) was ~30 million. Several important parameters related to testis function such as the number of Sertoli cells per testis and sperm production, correlated with the volume occupied by blood vessels. In conclusion, this study shows for the first time that mainly due to high Sertoli cell efficiency and seminiferous tubule volume density, spermatogenic efficiency in goats is the highest among the domestic mammals already investigated.

Keywords: testis, morphometry, Sertoli cell, Leydig cell, sperm production, goats

Introduction

The goat is economically a very important domestic mammal. However, very few data are available in the literature for testis function and the spermatogenic process in this species (Courtens and Loir, 1981; Bilaspuri and Guraya, 1984; Oke et al., 1984; Onyango et al., 2000), mainly data concerning the testis morphometry obtained with high-quality light microscopy, using glutaraldehyde fixed and plastic embedded tissue. Recent work from our laboratory found that the duration of the seminiferous epithelium cycle in goats is 10.6 days (França et al., 1999). This data allowed, for the first time, to estimate the daily sperm production per gram of testis in this species and to compare its spermatogenic efficiency with other mammals.

The number of germ cells supported by a single Sertoli cell is the best reflection of the functional efficiency of this cell and is usually highly correlated with spermatogenic efficiency (daily sperm production per testis gram) (Russell and Peterson, 1984; França and Russell, 1998). In terms of efficiency of sperm production per unit area of seminiferous tubule, the most meaningful relationship is the number of spermatids per Sertoli cell (Russell and Peterson, 1984). Besides that, data available in the literature show that spermatogenic efficiency is also highly correlated with seminiferous tubules volume density, number of Sertoli cells per testis gram, and spermatogenic cycle length

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(Russell et al., 1990, Sharpe, 1994, Neves, 2001; Leal, 2004). In this regard, daily sperm production can be accurately obtained from the total number of Sertoli cells per testis and the number of spermatids per Sertoli cell (França and Russell, 1998; França and Godinho, 2003). In the present investigation, quantitative data related to the Sertoli cell, Leydig cell and daily sperm production were obtained for the first time in goats.

Materials and Methods

Animals

Four sexually-mature Alpine bucks (*Capra hircus*; 25.3 ± 1.0 months old) composing an homogeneous group and with 59.3 ± 5.4kg of mean body weight were utilized. All animals were in good health and of proved fertility since they were being used as sires in a specialized goat farm. After both the testes and epididymides were removed they were perfused-fixed with 4% buffered glutaraldehyde for 25-30 minutes, via a needle through the testicular artery. Following this, testes were trimmed out from the epididymis, weighed and cut longitudinally in order to obtain cross-sections of the seminiferous tubules. Tissue samples, 1-3mm thick, were taken near the tunica albuginea. These fragments were embedded in plastic (glycol methacrylate). Sections of 4µm thickness were stained with toluidine blue and utilized for histological and morphometric evaluation of the testis.

Before surgery, all animals received i.m. injection of 1mL of Rompum (Bayer) per 100kg of body weight and local scrotal anaesthesia (Pearson). All surgical procedures were performed by a veterinarian and followed approved guidelines for the ethical treatment of animals.

Testis morphometry

The tubular diameter and the height of seminiferous tubule epithelium were measured at 100X magnification using an ocular micrometer calibrated with a stage micrometer. At least thirty tubular profiles that were round or nearly round were chosen randomly, and measured for each animal. The epithelium height was obtained in the same tubules utilized to determine tubular diameter. The volume densities of various testicular tissue components were determined by light microscopy using a 441-intersection grid placed in the ocular of the light microscope. Fifteen fields chosen randomly (6,615 points) were scored for each animal at 400X magnification. Artifacts were rarely seen and were not considered in the total number of points utilized to obtain volume densities. Points were classified as one of the following: seminiferous tubule, comprising tunica propria, epithelium and lumen; Leydig cell; blood and lymphatic vessels; and connective tissue.

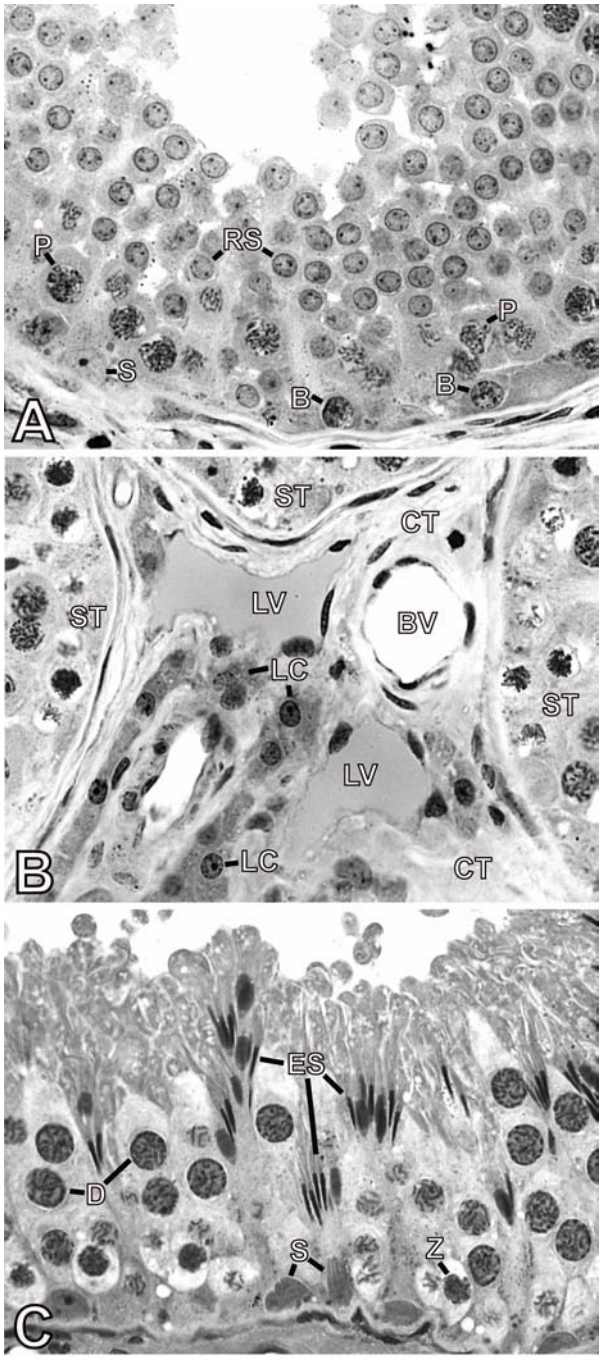

Figure 1. (A) Seminiferous tubule cross-section in goat at stage 1 of the cycle. Depicted in this figure are type B (B) spermatogonia; pachytene primary spermatocytes (P); round spermatids (RS); and Sertoli cells (S). (B) Intertubular compartment in goats showing lymphatic (LV) and blood vessels (BV), Leydig cells (LC), and connective tissue (CT). Seminiferous tubules are also shown (ST). (C) This figure, at stage 3 of seminiferous epithelium cycle, illustrates elongated spermatids bundles (ES) among diplotene primary spermatocytes (D) and oriented toward Sertoli cell nucleus (S). All figures are stained with Toluidine blue. Magnification at 400x.

The volume of each component of the testis was determined as the product of the volume density and testis volume. For subsequent morphometric calculations, the specific gravity of testis tissue was considered to be 1.0. To obtain a more precise measure of testis volume, the testis capsule plus mediastinum (~10%) obtained in the same breed (Alpine bucks; Becker-Silva, 2000) were excluded from the testis weight. The total length of seminiferous tubule (meters) was obtained by dividing seminiferous tubule volume by the squared radius of the tubule times the pi value (Johnson and Neaves, 1981).

Cell counts and cell numbers

All germ cells nuclei and Sertoli cell nucleoli present at stage 1 of the cycle (Fig.1A), characterized according to the tubular morphology system (França et al., 1999) were counted in ten round or nearly round seminiferous tubule cross-sections, chosen at random, for each animal. These counts were corrected for section thickness and nucleus or nucleolus diameter according to Abercrombie (1946), as modified by Amann (1962). For this purpose, ten 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 Sertoli cells was determined from the corrected counts of Sertoli cell nucleoli per seminiferous tubule cross-sections and the total length of seminiferous tubules according to Hochereau-de Reviers and Lincoln (1978). The daily sperm production (DSP) per testis and per gram of testis (spermatogenic efficiency) were obtained according to the formula developed by França (1992) as follow: DSP = Total number of Sertoli cells per testis x the ratio of round spermatids to Sertoli cells at stage 1 x stage 1 relative frequency (%)/stage 1 duration (days).

Individual volume of the Leydig cell was obtained from nucleus volume and the proportion between nucleus and cytoplasm. Because the Leydig cell nucleus in goats is spherical, its nucleus volume was obtained from the knowledge of the mean nuclear diameter. For this purpose, thirty nuclei showing evident nucleolus had their diameter measured for each animal. Leydig cell nuclear volume was expressed in μm^3 and obtained by the formula $4/3\pi R^3$, were R = nuclear diameter/2. To calculate the proportion between nucleus and cytoplasm a 441-point square lattice was placed over the sectioned material at 400X magnification. One thousand points over Leydig cells were counted for each animal. The number of Leydig cell per testis was estimated from the Leydig cell individual volume and the volume occupied by Leydig cell in the testis parenchyma.

Statistical analyses

All data are presented as the mean ± SEM. Analysis of correlation was carried out using the program STATISTICA for windows (StatSoft, Inc., Tulsa, OK). The significance level considered was $p<0.05$.

Results

Biometric data and testis volume density

The mean testis weight found for the four sexually mature goats was approximately 100 grams, providing a gonadosomatic index (testes mass divided by body weight) of approximately 0.35% (Table 1). The volume density of seminiferous tubules and Leydig cells was approximately 88% and 1.5%, respectively (Table 1). It means that Leydig cells occupy only ~10% of the intertubular compartment. In this compartment, Leydig cells are organized in clusters and the irregularly shaped lymphatic vessels are randomly distributed (Fig.1B). The testis weight correlated significantly and positively (r = 0.99) with the percentage and volume occupied by blood vessels in the testis parenchyma.

Table 1. Biometric and morphometric data in sexually mature goats (Mean ± SEM).

Body weight (kg)	59 ± 6
Testis weight (g)	101 ± 6*
Gonadosomatic index (%)	0.35 ± 0.02
Testis parenchyma volume density (%):	
Seminiferous tubule	87.7 ± 1.1
Tunica propria	3.0 ± 0.6
Seminiferous epithelium	75.4 ± 1.0
Lumen	9.3 ± 0.9
Intertubular compartment	12.3 ± 1.1
Leydig cell	1.4 ± 0.3
Connective tissue	8.0 ± 0.7
Blood vessels	2.2 ± 0.2
Lymphatic vessels	0.7 ± 0.2
Tubular diameter (μm)	237 ± 3
Seminiferous epithelium height (μm)	78 ± 2
Tubular length per gram of testis (meters)	20 ± 0.6
Total tubular length per testis (meters)	1,829 ± 130

Mean testis weight (Right testis + left testis / 2).

The mean tubular diameter and epithelium height was 237µm and 78µm, respectively (Table 1). Based on the volume of the testis parenchyma (testis weight minus tunica albuginea plus mediastinum weight), and the volume occupied by seminiferous tubules in the testis and the tubular diameter, 20 meters of seminiferous tubules were found per testis gram, and the total tubular length per testis was ~1,830 meters (Table 1). The total length of seminiferous tubule was significantly and positively correlated with the blood vessels volume (r = 0.95). Also, similar correlation was observed between seminiferous epithelium volume and blood vessels volume (r = 0.99).

Testis morphometry

Leydig cell nuclear volume and cell size were approximately 170µm^3 and 780µm^3, respectively (Table 2). The numbers of Leydig and Sertoli cells per testis and per gram of testis were ~18 and ~21 millions, respectively, whereas these figures per testis were ~1.7 and ~2 billions (Table 2). The total number of Sertoli cells showed highly and significant positive correlation with testis weight (r = 0.98), the percentage and volume occupied by blood vessels in the testis parenchyma (r = 1.0), and the total of seminiferous tubule (r = 0.98) and seminiferous epithelium volume (r = 0.99). On the other hand, the total number of Leydig cells was significantly and positively correlated with the percentage (r = 0.98) and volume (r = 0.99) occupied by lymphatic vessels in the testis parenchyma.

The meiotic index, measured as the number of round spermatids produced per pachytene primary spermatocytes, was 2.8 ± 0.3. This result shows that 30% of cell loss occurs during the two meiotic divisions. The Sertoli cell efficiency in goats, estimated from the total number of germ cells and spermatids per Sertoli cell at stage 1 (Fig. 1A) of the seminiferous epithelium cycle was 25 ± 2 and 15.4 ± 1.4, respectively. Fig.1C illustrates elongate spermatids bundles associated with a single Sertoli cell. Approximately thirty-seven primary spermatocytes at pachytene phase were found per each type A spermatogonia present at stage 1 of the cycle. The daily sperm production per testis and per gram of testis in goats was approximately 30 million and 2.8 billion, respectively (Table 2). Daily sperm production per testis was highly and positively correlated with several parameters such as testis weight (r = 0.98), percentage and volume of blood vessels (r = 0.96), number of Sertoli cells per tubular cross-section (r = 0.97) and per testis (r = 0.96) or testis gram (r = 0.96), and seminiferous epithelium volume (r = 0.99).

Discussion

To our knowledge, this is the first report to perform a more comprehensive morphometric and functional investigation of the testis in goats, showing not only Sertoli and Leydig cell numbers per testis, but also the spermatogenic efficiency for this species.

Table 2. Leydig and Sertoli cell morphometry and daily sperm production (Mean ± SEM).

Leydig cell nuclear diameter (µm)	6.9 ± 0.1
Leydig cell volume (µm^3)	775 ± 75
Nuclear volume (µm^3)	169 ± 10
Cytoplasm volume (µm^3)	606 ± 69
Leydig cell number per gram of testis (x10^6)	18.3 ± 5.5
Leydig cell number per testis (x10^9)	1.72 ± 0.5
Sertoli cell number per gram of testis (x10^6)	21.4 ± 1.8
Sertoli cell number per testis (x10^9)	1.98 ± 0.27
Daily sperm production per gram of testis (x10^6)	30.3 ± 0.3
Daily sperm production per testis (x10^9)	2.77 ± 0.14

The value found for the gonadosomatic index in goats (~0.4) is much lower than that observed for rams (0.8). However, the former values were much higher than those showed for other domestic ruminants such as bulls (0.1) and buffalos (0.04) (Kenagy and Trombulack, 1986; França and Russell, 1998). This finding shows that goats has a fairly good potential for sperm production. Seminiferous tubules comprise the main compartment of the testis and occupies from ~70% to ~90% of testis parenchyma in most mammals investigated (Russell et al., 1990; França and Russell, 1998). In this regard, as compared to other mammalian species, the value found for the percentage of seminiferous tubules in the goats herein studied is in the upper level of that range, particularly when only domestic mammals are considered (Table 3). However, data available for this parameter in the literature for other goat breeds is around 80-85% (Oke et al., 1984; Yadav and Sharma, 1994). Also, the value observed for tubular diameter in the present investigation is about 15% higher than that found in other report for domestic goats (Oke et al., 1984; França and Russell, 1998; Yadav and Sharma, 1994; Nishimura et al., 2000) and is in the range cited (180 to 350µm) for most mammals

investigated (Roosen-Runge, 1977; Setchell et al., 1994). However, it should be mentioned that approximately 15% of shrinkage of testicular tissue due to paraffin embedding is usually expected, whereas this shrinkage of tissue embedded in plastic resins, as performed in the present work, is very low or negligible (França and Russell, 1998). Therefore, besides expected variation for different breeds or strains (Okwun et al., 1996), at least partially the variation observed in tubular diameter for goats can be attributed to the methodological approaches utilized for tissue preparation before analysis.

Table 3. Comparative data on testis composition, Sertoli cell efficiency, number of Sertoli cells per testis gram, cycle length, and daily sperm production per testis gram (DSP) in domestic mammals*.

Species	Seminiferous tubules (%)	Leydig cell (%)	Number of Sertoli cells per testis gram ($x10^6$)	Number of spermatids per Sertoli cell	Cycle length (days)	DSP per testis gram ($x10^6$)
Rabbit	87	2.2	25	12.2	10.9	26
Boar	83	10.0	20	12.4	9.0	25
Ram	80-87	1.1-3.2	8-12	-	10.5	20-25
Stallion	73	18.4	28	8.7	12.2	19
Cat	88	6.0	32	5.1	10.4	16
Bull	81	4.8	29	8.0	13.3	13

See literature review in França and Russell, 1998; and França and Godinho, 2003

In general, 10 to 15 meters of seminiferous tubules are found per gram of testis parenchyma (França and Russell, 1998; Setchell et al., 1994). Probably due to the fact that the tubular diameter in goats is not very high and that almost 90% of seminiferous tubules are found in testis parenchyma, approximately twenty meters of tubules per gram are observed in this species (Yadav and Sharma, 1994). However, because the testis size in goats studied by Yadav and colleagues was approximately 50% smaller, the total tubular length in their study represents less than 60% of the value found in the present work.

To our knowledge, this is the first report showing Leydig cell volume density, individual volume, and the number of Leydig cells per testis in goats. Compared to most mammals investigated, both Leydig cell size and volume density in goats are very low (Table 3) (Russell, 1996; França and Russell, 1998). The organization of Leydig cells in goats, where the lymphatic vessel is located nearby clusters of Leydig cells, is similar to the type II pattern previously reported (Fawcett et al., 1973). Besides producing testosterone, Leydig cells secrete other steroids and pheromones that are important for other reproductive functions such as sexual behavior and maintenance of sexual accessory gland function. For instance, as shown for goats (Goyal et al., 1997), recent studies demonstrated that estrogen is important for the male reproductive tract function (reviewed in Hess et al. 2001). It has been also established in the literature that Leydig cell individual volume is correlated with the amount of smooth endoplasmic reticulum and with its capacity to secrete testosterone (Ewing et al., 1979; Zirkin et al., 1980). However, it remains to be elucidated why very high variation in Leydig cell organization in the testis is observed in mammals (Fawcett et al., 1973; Russell, 1996). Similarly, there is very little knowledge regarding the reason why dramatic variation is observed for Leydig cell volume density (~1% in rams to ~35% in capybaras), Leydig cell individual volume (~400µm^3 in rams to ~5,000µm^3 in horses) (Johnson and Neaves, 1981; Lunstra and Schanbacher, 1988) and Leydig cell number per gram of testis (~6 millions in guinea pigs to ~160 millions in wild boars) (França and Russell, 1998; Russell, 1996; Almeida, 2002).

Apoptosis occurs normally during specific steps of germ cell development (Roosen-Runge, 1973; Blanco-Rodriguez, 1998) and can be estimated comparing the ratio of germ cell numbers before and after a given developmental step (França and Russell, 1998). In mammals, only 2 or 3 of 10 spermatozoa are produced from differentiated type A1 spermatogonia and the highest level of cell degeneration occurs during the spermatogonial proliferative phase and during meiosis (França and Russell, 1998). Therefore, similar to what is found for most mammals (Roosen-Runge, 1973; França and Russell, 1998), the meiotic index observed for goats in the present work showed that 30% of cell loss occurred during the two meiotic divisions. However, other report (Bilaspuri and Guraya, 1984) for goats showed only ~15% of germ cell loss during this phase of spermatogenesis. In the present study, the kinetics of spermatogonia was not investigated. Compared with other mammals such as mice, rats and pigs (França and Russell, 1998; De Rooij and Russell, 2000),

the number of primary spermatocytes at preleptotene/leptotene per each type A spermatogonia present at stage 1 suggest that in goats six generations of differentiated spermatogonia are present (Bilaspuri and Guraya, 1984).

Because each Sertoli cell supports a limited number of germ cells in a species-specific manner (Russell and Peterson, 1984; França and Russell, 1998), it is currently accepted that the number of Sertoli cells established during testis development determines the rate of sperm production in sexually mature animals (Orth et al., 1988; Hess et al., 1993; França et al., 2000). In addition, reports that spermatogenic efficiency is usually positively correlated with the number of germ cells supported by each Sertoli cell (Russell and Peterson, 1984; Sharpe, 1994; França and Russell, 1998). The volume density of seminiferous tubules, the length of spermatogenic cycle, the number of spermatogonial generations, the rate of germ cell loss during spermatogenesis, the number of Sertoli cells per gram of testis and the size of Sertoli cells are also important parameters for the determination of spermatogenic efficiency (Johnson, 1991; França and Russell, 1998; Johnson et al., 2000; França et al., 2002). Paradoxically, the higher the Sertoli cell size is, the lower its support capacity for germ cells, because this cell would occupy too much space in the seminiferous epithelium (Russell et al., 1990). Also, as observed for cats (França and Godinho, 2003) higher number of Sertoli cells per gram of testis among individuals from the same species correlates negatively with tubular diameter and, consequently, with the number of germ cells per tubule cross-section. Although not significant, similar trend was observed in the present work for goats.

The number of Sertoli cells per gram of testis found for goats in the present study is situated in an intermediate level compared to the values found for most mammalian species investigated (Russell et al., 1990; França and Russell, 1998). However, Sertoli cell efficiency in goats is one of the highest among mammals already investigated (Russell and Peterson, 1984; Sharpe, 1994; França and Russell, 1998; França et al., 2002). For this reason, and due to the high seminiferous tubule volume density and the fact that each seminiferous epithelium cycle length in goats is not long (10.6 days; França et al., 1999), spermatogenic efficiency found for this species is very high particularly for domestic mammals (Table 3).

An interesting finding in the present work was the consistently significant correlations between the volume occupied by blood vessels and the parameters related to testis function such as testis weight, total tubular length, seminiferous epithelium volume, number of Sertoli cells per testis, and spermatogenic efficiency. On the other hand, Leydig cells are more correlated with the volume occupied by lymphatic vessels. To our knowledge, although the number of animals utilized in the present study is low, this is the first report showing these functional relationships in the testis.

In conclusion, based on the number of spermatids found per Sertoli cell in goats, this cell is the most efficient among mammals so far studied. The association of this data with the values found for seminiferous tubules volume density in the testis parenchyma and the cycle length observed previously for goats, resulted in a very high spermatogenic efficiency for this species, being this efficiency the highest among domestic mammals already investigated.

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References

Abercrombie M. 1946. Estimation of nuclear populations from microtome sections. *Anat Rec*, 94:238-248.

Almeida FFL. 2002. *Testis structure and function in sexually mature wild boars* (*Sus scrofa scrofa*) [in Portuguese]. Belo Horizonte, Brazil: Federal University of Minas Gerais. Dissertation.

Amann RP. 1962. Reproductive capacity of dairy bull. III. The effect of ejaculation frequency, unilateral vasectomy and age on spermatogenesis. *Am J Anat,* 110:49-67.

Becker-Silva SC. 2000. *Seminal and histological characterization during testis development of Saanen goats raised in intensive system* [in Portuguese]. Belo Horizonte, Brazil: Federal Univ. of Minas Gerais. Thesis.

Bilaspuri GS, Guraya SS. 1984. The seminiferous epithelial cycle and spermatogenesis in goats (*Capra hircus*). *J Agric Sci, Camb,* 103, 359-368.

Blanco-Rodríguez J. 1998. A matter of death and life: the significance of germ cell death during spermatogenesis. *Int J Androl*, 21:236-248.

Courtens J-L, Loir M. 1981. A cytochemical study of nuclear changes in boar, bull, goat, mouse, rat, and stallion spermatids. *J Ultrastruc Res*, 74: 327-340.

De Rooij DG, Russell LD. 2000. All you wanted to know about spermatogonia but were afraid to ask. *J Androl*, 21:776-798.

Ewing LL, Zirkin BR, Cochran RC, Kromann N, Peters C, Ruiz-Bravo N. 1979. Testosterone secretion by rat, rabbit, guinea pig, dog, and hamster testes perfused in vitro: correlation with Leydig cell mass. *Endocrinology*, 105:1135-1142.

Fawcett DW, Neaves WB, Flores MN. 1973. Comparative observations on intertubular lymphatic and the organization of the interstitial tissue of the mammalian testis. *Biol Reprod.,* 9:500-532.

França LR. 1992. Daily sperm production in Piau boars estimated from Sertoli cell population and Sertoli cell index. *In*: Proceedings of the 12th International

Congress on Animal Reproduction a7nd Artificial In semination, 1992, The Hague, The Netherlands: ICAR. v.4, p.1716-1718.

França LR, Godinho CL. 2003. Testis morphometry, seminiferous epithelium cycle length, and daily sperm production in domestic cats (*Felis catus*). *Biol Reprod,* 68:1554-1561.

França LR, Russell LD. 1998. The testis of domestic animals. In: Martínez F, Regadera J (Eds.). *Male reproduction: a multidisciplinary overview*. Madrid, Spain: Churchill Livingstone. p.197-219.

França LR, Becker-Silva SC, Chiarini-Garcia H. 1999. The length of the cycle of seminiferous epithelium in goats (*Capra hircus*). *Tissue Cell*, 31:274-280.

França LR, Russell LD, Cummins JM. 2002. Is human spermatogenesis uniquely poor? *ARBS Ann Rev Biomed Sci*, 4:19-40.

França LR, Silva Jr VA, Chiarini-Garcia H, Garcia SK, Debeljuk L. 2000. Cell proliferation and hormonal changes during postnatal development of the testis in the pig. *Biol Reprod*, 63:1629-1636.

Goyal HO, Bartol FF, Wiley AA, Neft CW. 1997. Immunolocalization of receptors fpr androgen and estrogen in male caprine reproductive tissues: unique distribution of estrogen receptors in efferent ductile epithelium. *Biol Reprod*, 56:90-101.

Hess RA, Bunick D, Bahr J. 2001. Oestrogen, its receptors and function in the male reproductive tract – a review. *Mol Cell Endocr*, 178:29-38.

Hess RA, Cooke PS, Bunick D, Kirby JD. 1993. Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli cell and germ cell number. *Endocrinology*, 132:2607-2613.

Hochereau-de Reviers MT, Lincoln GA. 1978. Seasonal variation in the histology of the testis of the red deer, Cervus elephus. *J Reprod Fertil Suppl,* 54:209-213.

Johnson L. 1991. Spermatogenesis. Cupps PT (Ed.). *Reproduction in domestic animals*. 3rd. ed. New York: Academic Press. p.173-219.

Johnson L, Neaves, WB. 1981. Age-related changes in the Leydig cell population, seminiferous tubules and sperm production in stallions. *Biol Reprod,* 24:703-712.

Johnson L, Varner DD, Roberts ME, Smith TL, Keillor GE, Scrutchfield WL. 2000. Efficiency of spermatogenesis: a comparative approach. *Anim Reprod Sci*, 60-61:471-480.

Kenagy GJ, Trombulak SC. 1986. Size and function of mammalian testes in relation to body size. *J Mamm*, 67:1-22.

Leal MC. 2004. *Morphometric and functional analyses of testis and spermatogenic efficiency in the marmoset (C. penicillata)* [in Portuguese]. Belo Horizonte, Brazil: Federal University of Minas Gerais. Thesis.

Lunstra DD, Schanbacher BD. 1988. Testicular function and Leydig cell ultrastructure in long-term bilaterally cryptorchid rams. *Biol Reprod,* 38:145-158.

Neves ES. 2001. *Comparative study of the testis structure and spermat ogenic process in donkeys (Equus asinus) and mules (Equus mulus mulus)* [in Portuguese]. Belo Horizonte, Brazil: Federal University of Minas Gerais. Dissertation.

Nishimura S, Okano K, Yasukouchi K, Gotoh T, Tabata S, Iwamoto H. 2000. Testis developments and puberty in the male Tokara (Japanese native) goat. *Anim Reprod Sci*, 64:127-131.

Oke BO, Ogwuegbu SO, Akusu MO. 1984. Morphometric study of the testis of the west African Dwarf goat. *Bull Anim Hlth Prod S Afr*, 32:57-60.

Okwun OE, Igboeli G, Lunstra DD, Ford JJ, Johnson L. 1996. Testicular composition, number of A spermatogonia, germ cells ratio, and number of spermatids in three different breeds of boar. *J Androl,* 17:301-309.

Onyango DW, Wango EO, Otiang´a-Owiti GE, 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 JM, Gunsalus GL, Lampert AA. 1988. Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. *Endocrinology*, 122: 787-794.

Roosen-Runge EC. 1973. Germinal cell loss in normal metazoan spermatogenesis. *J Reprod Fertil*, 35:339-348.

Roosen-Runge EC. 1977. *The process of spermatogenesis in animals*. Cambridge, UK: University Press.

Russell LD. 1996. Mammalian Leydig cell structure. In: Payne AH, Hardy MP, Russell LD (Eds.). *The Leydig cell.* Vienna, IL: Cache River Press. p 43-96.

Russell LD, Peterson RN. 1984. Determination of the elongate spermatid-Sertoli cell ratio in various mammals. *J Reprod Fertil*, 70:635-64.

Russell LD, Ren HP, Sinha-Hikim I, Schulze W, Sinha-Hikim AP. 1990. A comparative study in twelve mammalian species of volume densities, volumes, and numerical densities of selected testis components emphasizing those related the Sertoli cell. *Am J Anat*, 188:21-30.

Setchell BP, Maddocks S, Brooks DE. 1994. Anatomy, vasculature, innervation, and fluids of the male reproductive tract. In: Knobil E, Neill JD (Eds.). *The physiology of reproduction*. 2nd ed, New York, NY: Raven Press. p 1063-1175.

Sharpe, R.M. 1994. Regulation of spermatogenesis. *In:* Knobil E,. Neil J.D. (Eds.). *The physiology of reproduction.* New York, NY: Raven Press. p.1363-1434.

Yadav SK, Sharma AK. 1994. Seminiferous length in normal buffalos-bulls and bucks. *Int J Anim Sci,* 9:293-296.

Zirkin BR, Ewing LL, Kromann N, Cochran RC. 1980. Testosterone secretion by rat, rabbit, guinea pig, dog, and hamster testes perfused in vitro: correlation with Leydig cell ultrastructure. *Endocrinology*, 107:1867-1874.