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Postnatal attributes of spermatogenesis and Sertoli cell efficiency of Assam goat (*Capra hircus*) in a tropical environment

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ABSTRACT

The present study was conducted in a total of eighteen numbers of healthy male Assam goats of age ranging from birth to 10 months. The goats were divided into six age groups viz. group-I (0-day), group-II (2 months), group-III (4 months), group-IV (6 months), group-V (8 months) and group-VI (10 months) consisting of 3 animals in each group. The mean nuclear diameters of the Sertoli cells registered an increase in their values with the advancement of the age of the male goats. The efficiency of spermatogonial mitosis as determined by estimating the ratio between pachytene primary spermatocytes and type A spermatogonia increased noticeably from 4 months of age (group-III) being the maximum in 10 months old goats. The overall rate of spermatogenesis as measured by recording the ratio between round spermatids and type A spermatogonia increased significantly between 6 and 8 months of age. Further, the overall Meiotic Index (ratio between round spermatids and pachytene primary spermatocytes) was recorded to be 2.45 ± 0.56, revealing about 38.75 percent cell apoptosis (loss) in Assam goats. The ratio between round spermatids and Sertoli cell nuclei increased in older goats and the Sertoli cell efficiency was found to be the maximum in 10-month-old goats as almost 16. Twelve spermatids were found for each Sertoli cell at this age.

Keywords: *Sertoli cell; efficiency of spermatogenesis; Assam goat; Meiotic index, Spermatids*

Introduction

Post-natal anatomical studies on the male genital system at various ages, particularly the testis and its tubular system are important to know the anatomical growth and development. The various measurements of different spermatogenic cells vary in different cycles of the seminiferous epithelium and within different species. The primary spermatocytes, round spermatids, elongated spermatids, and Sertoli cells formed 10.78 ± 1.0 %, 6.17 ± 0.5 %, 3.57 ± 0.1% and 1.67 ± 0.3%, respectively of the seminiferous epithelium (Oke *et al* 1984). The Sertoli cell was the major controller of testis development and efficiency of spermatogenesis (Hess *et al* 1993). The Sertoli cell was the major controller of testis development and efficiency of spermatogenesis (Hess *et al* 1993). Again, the FSH is considered to be the main mitogenic factor responsible for Sertoli cell divisions. The Sertoli cell number established during the pre-pubertal period determined the final testicular size and the number of sperm produced in sexually mature animals (Orth *et al* 1988). Germ cell loss (apoptosis) played an important role in seminiferous epithelial homeostasis by limiting the number of sperm cells produced which could be estimated by comparing the ratios between germ cells counted during specific steps of spermatogenesis (Johnson 1991).

Goat plays an important role in the socio-economic condition of the rural people as it is endowed with short generation intervals, higher rates of prolificacy and the ability to sustain on sparse vegetation and extreme climatic conditions. Goat rearing has tremendous potential in the North eastern states of India, particularly among the small and marginal farmers and landless laborers because of very low initial investment and adequate financial returns. This region has abundant natural grasses, pastures, shrubs and forests due to widespread rainfall. More than 85 percent of the population in this region is non-vegetarian and chevon is preferred by all as it has no religious taboo.

The present data generated in our study will contribute to a pattern of histological development of the Sertoli cells and efficiency of spermatogenesis at various ages in Assam goats, which is the first-ever study of its kind in this animal species.

Materials and Methods

A total of 72 male Assam goats varying in age from 0-day to 10 months were used in the present study. The animals were divided into six age groups *viz.* group-I (0-day), group-II (2 months), group-III (4 months), group-IV (6 months), group-V (8 months) and group-VI (10 months) consisting of twelve animals in each group. The age of the goats was estimated from birth records. Each animal was weighed using Spring Balance to record its body weight. The animals were sedated by giving an intramuscular injection of Siquil (Triflupromazine Hydrochloride) @ 1mg/Kg body weight and subsequently anaesthetized by administering intravenous injection of Intravel Sodium (Pentobarbital Sodium) @ 15 mg/Kg body weight (Hall et al., 2000). After induction of a proper level of anesthesia, the testicles were incised out by performing open method of castration as per standard protocol. Subsequently, the animals were given proper post-operative treatment and care. Tissue pieces were collected from three different regions of the testis *viz.*, upper, middle and lower, and subsequently fixed in Bouin's solution prepared as per (Luna 1968).

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All the tissues were processed for paraffin sections (Luna 1968) by alcohol-xylene method using ceder wood oil. Sections were cut at 5 µ thickness using a Rotary Microtome (Thermo, Germany) and stained with Haematoxylin & Eosin. Various micrometrical parameters were recorded as follows.

a) No. of Sertoli cells per cross-section of the seminiferous cord/tubule: 20 rounds seminiferous cords/tubules were considered at random per animal (Franca *et al* 2000).

b) Sertoli cell nuclear diameter: 20 Sertoli cell nuclei were measured to record their diameter per animal (Elizabeth *et al* 2002).

c) Number of various spermatogenic cells per seminiferous tubules:
Number of spermatogonia per seminiferous tubule
Number of primary spermatocytes per seminiferous tubule
Number of secondary spermatocytes per seminiferous tubule
Number of round spermatids per seminiferous tubule
Number of Sertoli cell nuclei per seminiferous tubule
These were counted in 10 round or nearly round cross sections of seminiferous tubules chosen at random per animal (Segatell *et al* 2004).

d) The ratios of various cells of the seminiferous tubules were estimated for determination of different functional aspects (Segatell *et al* 2004). These measurements were recorded in 10 numbers of randomly selected seminiferous tubules per animal as follows.

Ratio	Functional aspects
(i) Pachytene spermatocytes / Type A spermatogonia	To obtain an estimation of efficiency of spermatogonial mitosis
(ii) Round spermatids / Type A spermatogonia	To obtain an overall rate of spermatogenesis
(iii) Round spermatids / Pachytene spermatocytes	To obtain rate of germ cell apoptosis (loss) during meiosis (Meiotic Index)
(iv) Round spermatids / Sertoli cell nuclei	To estimate Sertoli cell efficiency

Results

The measurements of number of various cells per cross-section of the seminiferous tubule of the testis in the male goats at various ages under study were presented in Table 1. Each seminiferous tubule of the testes of 4-month-old kids contained an average number of 18.2 ± 1.28 type- A spermatogonia which increased gradually in older goats and ultimately raised to 20.8 ± 1.24 number of cells at 10 months of age. However, the variation in the number of these cells between different age groups was not significant (Table 2). Similarly, the mean number of type-B spermatogonia increased with advancing age in male goats which ranged from 9.4 ± 0.93 cells at 4 months of age to 11.35 ± 1.69 cells at 10 months of age. The highest number of this cell type was recorded in 10 months-old goats. However, the increase in number of type-B spermatogonia between various age groups was not found to be significant (Table 2). The average number of primary spermatocytes ranged from 22.4 ± 3.23 cells per cross section of seminiferous tubule at 4 months of age to 43.3 ± 4.40 at 10 months of age. Analysis of variance (Table 2) revealed a highly significant ($P<0.01$) increase of these cells between successive higher age groups. The number of primary spermatocytes increased significantly ($P<0.05$) between 4 and 6 months of age (groups III & IV) and 6 and 8 months of age (groups-IV & V). The average number of secondary spermatocytes per cross-section of seminiferous tubule ranged from 41.4 ± 5.93 at 6 months of age to 44.8 ± 2.46 cells at 8 months of age. Maximum number of these cells was recorded in 8-month-old goats. The variation in the number of these cells between different age groups was not found to be significant ($P<0.05$) (Table 2).

The average number of the round spermatids ranged from 52.8 ± 1.28 at 6 months of age to 125.6 ± 1.50 cells per seminiferous tubules at 10 months of age. Analysis of variance (Table 2) showed a highly significant ($P<0.01$) increase of these cells between the various age groups under study. There was a significant ($P<0.05$) increase of mean number of round spermatids between 6- and 8-months old bucks (groups IV & V). The mean number of elongated spermatids per cross-section of seminiferous tubule increased from 34.40 ± 1.99 cells at 6 months to 44.00 ± 1.52 cells at 8 months of age, thereafter the mean number of this cell decreased a little to 43.10 ± 1.32 cells at 10 months of age. Analysis of variance (Table 2) showed a highly significant ($P<0.01$) variation in number of elongated spermatids between different age groups under study.

The average number of Sertoli cells per cross section of the seminiferous tubule showed a fluctuating pattern (Fig. 1) at various ages in male Assam goats. The mean number of Sertoli cells was the maximum (17.0 ± 0.89) in day-old kids which showed an increasing pattern from 2 months of age (8.00 ± 0.71) up to 9.40 ± 1.21 at 8 months of age, and then it was slightly reduced to 9.21 ± 1.20 in 10-month-old goats. The variation in the number of Sertoli cells per cross-section of sex cord/seminiferous tubule between various age groups was not found to be significant (Table 2).

The mean ratios of different cells of the seminiferous epithelium in various age groups of male Assam goats have been presented in Table 3. The mean ratio between round spermatids and type-A spermatogonia (Fig. 2) could be recorded in the male goats from 6 months of age (group- IV) onwards. The ratio showed an increasing trend with the advancing age of the animals and varied from 5.62 ± 0.35 at 6 months of age to 11.07 ± 0.74 at 10 months of age. The variation of this ratio was found to be highly significant ($P<0.01$) between various age groups (Table 4). In the present study, the ratio between pachytene primary spermatocytes and the type- A spermatogonia showed a significant (Table 2) increasing pattern (Fig. 3) with the advancing age of the goats and ranged between 2.10 ± 0.34 to 3.35 ± 0.20 at 4 months and 10 months of age, respectively.

The mean ratio between round spermatids and pachytene primary spermatocytes was recorded in 6 months old goats onwards. This ratio showed a highly significant (P< 0.01) increase with the advancement of age of the goats (Fig. 4). The ratio increased significantly between 6 and 8 months of age (groups-IV & V). The mean ratio between round spermatids and Sertoli cell nuclei could be recorded in goats from 6 months onwards. This ratio increased with the advancement of age of the goats and ranged from 6.14 ± 1.11 at 6 month of age (group-IV) to 14.60 ± 2.79 at10 months of age (group-VI). Analysis of variance (Table 4) showed a highly significant (P<0.01) variation of this ratio between various age groups under study.

Discussion

The mean numbers of Type A & B spermatogonia per seminiferous tubule increased in the male kids in advancing age groups (Table 1). Again, the number of primary spermatocytes increased significantly (P<0.05) between 4, 6, and 8 months of age (groups- III, IV & V), which might be to aid formation of a greater number of progenitor cells from them during and just after puberty. The secondary spermatocytes showed their appearance in the testes at 6 months of age (group-IV) and their number in the seminiferous tubules did not vary significantly between the older age groups as the secondary spermatocytes appeared for a very transient period in the testicular tissue and immediately, they became transformed into the round spermatids (Dellmann 1988, Franca and Russel 1998). The mean number of the round and elongated spermatids increased significantly (P<0.5) between 6 and 8 months of age (groups- IV & V). However, the mean number of elongated spermatids showed a slight reduction in their number at 10 months of age (group-VI) which was not significant between the preceding age groups (Table 1). This minimum reduction in the number of elongated spermatids per seminiferous tubule in 10 months old goats (group-VI) might the part of the process approaching towards establishing an adult value of these cells in the testes of Assam goats at this age. In pigs, the number of germ cell per seminiferous cord/tubular cross section was very low from birth to 4 months of age. A very dramatic increase in various populations of germ cells per cross-section of seminiferous tubule occurred from 4 to 5 months of age but, the number of various germ cells showed a tendency to stabilize after 7 months of age (Franca *et al* 2000).

The mean number of Sertoli (Sustentacular) cells per cross-section of sex cord recorded in the male Assam goat kids at birth was the highest among all the age groups under study. The number of these cells was reduced at 2 month of age (group-II), remained almost same at 4 and 6 months of age (group-III & IV) then showed a slight rise in their number at 8 months of age (group-V). Subsequently, the mean number of Sertoli cells per cross-section of the seminiferous tubule reduced at 10 months of age. These findings were in close corroboration with earlier reports (Sinowatz and Amselgruber 1986) in cattle and in pigs (Franca *et al* 2000). Fernanda *et al* (2006) reported the presence of 6.6 Sertoli cells per seminiferous tubule in wild boar. The increased number Sertoli cells per cross section of the sex cord recorded in the present work in day-old kids might be the species-specific characteristics of goats which need further work in this aspect as no available literature could be traced to compare with the present findings.

Germ cell loss (apoptosis) played an important role in the seminiferous epithelial homeostasis by limiting the number of sperm cells produced which could be estimated by comparing the ratios between germ cells counted during specific steps of spermatogenesis (Johnson 1991). Estimation of ratio between pachytene primary spermatocytes and type A spermatogonia in the present work (Table 3) revealed that the efficiency of spermatogonial mitosis increased noticeably from 4 months of age onwards being the maximum in 10 months old goats (group-VI). The overall rate of spermatogenesis as measured by estimating the ratio between round spermatids and type A spermatogonia was recorded from 6 month of age (group-IV) onwards. The rate increased significantly (P<0.05) between 6- and 8-months old goats (groups-IV & V) (Table 4). Again, the Meiotic Index (ratio between round spermatids and pachytene primary spermatocytes) recorded in the present study in Assam goats ranged from 1.33 ± 0.02 at 6 months to 3.06 ± 0.16 in 10 months old goats denoting a decrease in germ cell loss during meiosis in older goats. The overall Meiotic Index in Assam goats was recorded as 2.45 ± 0.56, indicating that 38.75 percent of cell loss occurs during meiosis as one pachytene spermatocyte produces 4 round spermatids (Segatell *et al* 2004). This value of cell loss was similar to that observed for most mammalian species investigated (Roosen-Rung 1973, Franca and Russel 1998).

The efficiency of Sertoli cells increased from 6 (group-IV) to 10 month (group-VI) of age in Assam goats and the ratio of round spermatid and Sertoli cell nuclei showed a significant (P<0.05) increase between 6 and 8 months of age (groups-IV & V). The Sertoli cell efficiency was found to be the maximum in 10 months-old Assam goats as almost 16. Twelve spermatids were found for each Sertoli cell. The overall number of 13 spermatids was found for each Sertoli cell in all the 3 age groups studied (*viz.* groups- IV, V & VI) in the present work. A similar value was also recorded in Gerbil (Segatell *et al* 2004). However, the present value for efficiency of Sertoli cell was relatively high as compared with other mammalian species (Wing 1982, Franca and Russel 1998).

The volume densities of different spermatogenic cells of the testis increased with advancing age, thereby occupying more volumes in the seminiferous epithelium needed for enhanced physiological functions of the testes in pubertal and post-pubertal male Assam goats. Relevant information pertaining to this parameter was found to be very scant in the available literature. However, Franca *et al* (2000) reported similar findings during post-natal development in pigs.

Conclusion

The ratio between round spermatids and Sertoli cell nuclei increased in older goats and the Sertoli cell efficiency was found to be the maximum in 10-month-old goats as almost 16. Twelve spermatids were found for each Sertoli cell at this age.

Conflict of interest statement

The authors declare no competing interests.

Table 1: Number (Mean ± S.E.) of different cells per cross section of the seminiferous cord/tubule of testis of male assam goats at different ages

Age groups	Type A spermatogonia	Type B Spermatogonia	Primary spermatocytes	Secondary Spermatocytes	Round Spermatids	Elongated Spermatids	Sertoli Cells
0-Day	0.00	0.00	0.00	0.00	0.00	0.00	17.00^{abcdef}± 0.89
2- months	0.00	0.00	0.00	0.00	0.00	0.00	8.00^{a} ± 0.71
4- months	18.20^{a} ± 1.28	9.4^{a} ± 0.93	22.40^{a} ± 3.23	0.00	0.00	0.00	8.60^{ab} ± 1.36
6- months	10.00^{ab} ± 0.95	10.10^{ab} ± 1.89	32.60^{b} ± 2.30	41.40^{a} ± 5.93	52.8^{a} ± 1.28	34.40^{a} ± 1.99	8.60^{abc} ± 1.20
8- months	19.40^{abc} ± 1.03	11.30^{abc} ± 1.75	42.80^{c} ± 2.98	44.80^{abc} ± 2.46	123.6^{b} ± 1.69	44.00^{bc} ± 1.52	9.40^{abcde} ± 1.21
10- months	20.80^{abcd} ± 1.24	11.35^{abcd} ± 1.69	43.30^{cd} ± 4.40	44.50^{ab} ± 0.87	125.6^{bc} ± 1.50	43.10^{b} ± 1.32	9.21^{abcd} ± 1.20
Total	**19.35 ± 0.54**	**10.69 ± 1.73**	**35.28 ± 5.12**	**43.57 ± 2.08**	**100.57 ± 23.94**	**40.50 ± 2.95**	**10.14 ± 0.37**

Means bearing similar superscript in a column do not differ significantly.

Table 2: Analysis of variance of number of different cells of seminiferous epithelium per cross section of seminiferous cord/tubule showing significance of difference between various age groups of male assam goats

Parameters	Source	d.f.	S.S.	M.S.S.
Type A Spermatogonia	Age group	5	1263.07	252.613^{NS}
	Error	24	275.600	11.4833
Type B Spermatogonia	Age group	5	1571.87	314.373^{NS}
	Error	24	129.600	5.400
Primary Spermatocytes	Age group	5	6128.57	1225.71^{**}
	Error	24	964.800	40.200
Secondary Spermatocytes	Age group	5	10801.50	2160.30^{NS}
	Error	24	1204.00	50.1667
Round Spermatids	Age group	5	79438.30	15887.70^{**}
	Error	24	189.997	7.9165
Elongated Spermatids	Age group	5	9020.70	1804.14^{**}
	Error	24	210.00	8.75
Sertoli cells	Age group	5	16.1667	3.2333^{NS}
	Error	24	150.800	6.2833

*d.f. = Degree of freedom, S.S. = Sum of squares, M.S.S. = Mean sum of squares, F = Variance rates, ^{NS} = Non-significant, ** = P<0.01*

Table 3: Ratio (mean ± s.e.) of different spermatogenic cells of the seminiferous epithelium of testis of male assam goats at different ages

Age Groups	R.S./Type A Spermatogonia	Pachy. /Type A Spermatogonia	R.S./Pachy.	R.S./Sertoli cells
0-Day	0.00	0.00	0.00	0.00
2- months	0.00	0.00	0.00	0.00
4- months	0.00	2.10^{a} ± 0.34	0.00	0.00
6- months	5.62^{a} ± 0.35	2.88^{b} ± 0.28	1.3^{a} ± 0.02	6.14^{a} ± 1.11
8- months	10.54^{b} ± 0.54	3.33^{bc} ± 0.17	2.95^{b} ± 0.04	13.15^{b} ± 2.44
10- months	11.07^{bc} ± 0.74	3.35^{bcd} ± 0.20	3.06^{bc} ± 0.16	14.60^{bc} ± 2.79
Total	**9.21 ± 1.38**	**2.89 ± 0.15**	**2.45 ± 0.56**	**11.30 ± 2.66**

Means bearing similar superscript in a column do not differ significantly.
N.B. R.S.= Round Spermatids, Pachy = Pachytene Primary Spermatocytes

Table 4.: Analysis of variance of ratio between different cells of the seminiferous epithelium showing significance of difference between various age groups of male assam goats

Parameters	Source	S.S.	M.S.S.
Ratio between Round Spermatids and type A Spermatogonia	Age group	376.827	75.3654**
	Error	39.8658	1.661
Ratio between Pachytene Primary Spermatocytes and type A Spermatogonia	Age group	39.9257	7.9852**
	Error	6.6749	0.2781
Ratio between Round Spermatids and Pachytene Primary Spermatocytes	Age group	45.8683	9.1737**
	Error	0.5638	0.2349
Ratio between Round Spermatids and Sertoli cells	Age group	1188.15	237.630**
	Error	325.841	13.5767

*S.S. = Sum of squares, M.S.S. = Mean sum of squares, F = Variance rates, ** = P<0.01*

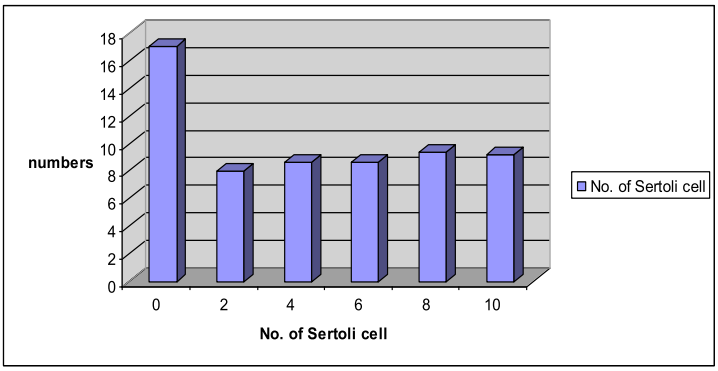

Fig. 1. Number of Sertoli cells (X 10^9) per cross section of the seminiferous tubule of the testes in Assam goats at different ages.

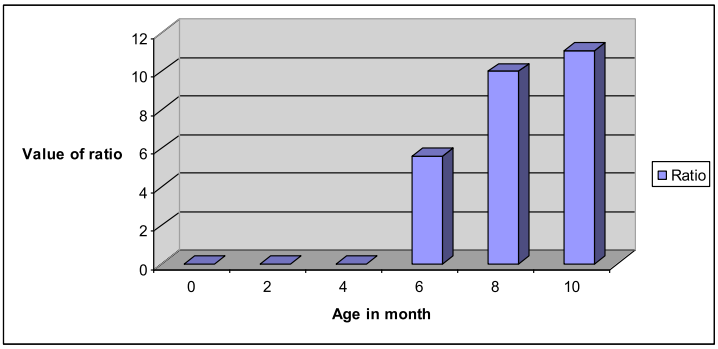

Fig. 2. Ratio between Round Spermatids and type-A spermatogonia of the seminiferous tubule of the testes in Assam goats at different ages.

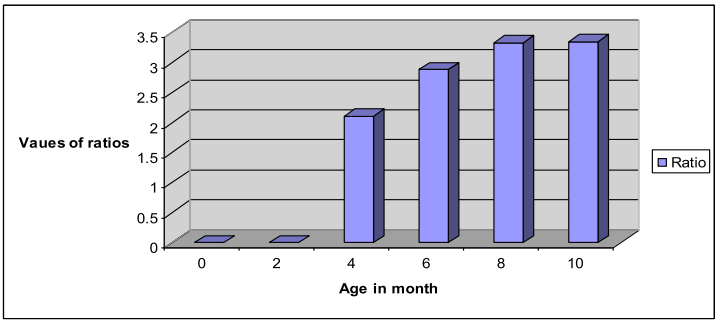

Fig. 3. Ratio between pachytene primary spermatocytes and type-A spermatogonia of the seminiferous tubule of the testes in Assam goats at different ages.

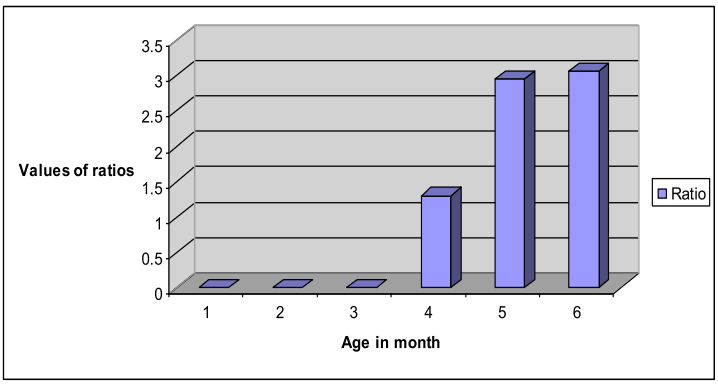

Fig. 4. Ratio between round spermatids and pachytene primary spermatocytes of the seminiferous tubules of the testes in Assam goats at different ages.

References

1. Dellmann, H. (1988). Endocrine System. In: Textbook of Veterinary Histology. Dellmann, H. and Brown, E.M. (eds.), Lea and Febiger, Philadelphia, 371-399.

2. Fernanda, F. L., Almeida, M., Leal, C., & Franca, L. R. (2006). Testis morphometry, duration of spermatogenesis, and spermatogenic efficiency in the wild boar (*Sus scrofa scrofa*). Biology of Reproduction *75*(5), 792-799.

3. Franca, L. R., & Russel, L. D. (1998). The testis of domestic animals. In: Martinez, F.; Regadera, J. (Eds.), Male Reproduction, A multidisciplinary overview. 1st edn, Madrid: Churchill Livingstone, 197-219.

4. Franca, L. R., Silva, V. A., Garcia, H. C., Garcia, S. K., & Debeljuk, L. (2000). Cell proliferation and hormonal changes during postnatal development of the testis in the pig. Biology of Reproduction *63*(6), 1629-1636.

5. Hall, L. W., Clarke, K. W., & Trim, C. M. (2000). Wright's Veterinary Anesthesia and Analgesia. 10th edn., ELBS and Baillierre Tindall, London, 172-175.

6. Hess, R. A., Cooke, P. S., Bunick, D., & Kirby, J. D. (1993). Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli cell and germ cell number. Endocrinology *132*(6), 2607-2613.

7. Johnson, L. (1991). Seasonal differences in equine spermatogenesis. Biology of Reproduction *44*, 284-291.

8. Luna, L. G. (1968). Manual of histological staining methods of Armed Forces Institute of Pathology, 3rd Edn. McGraw Hill Book Co., New York, 153-173.

9. Oke, B. O., Ugwuegbu, S. O. & Akusu, M. O. (1984). Morphometric study of the testis of the West African Dwarf goat. Bulletin of Animal Health and Production in Africa *32*, 57-60.

10. Orth, J. M., Gunsalus, G. L. & Lamperti, A. A. (1988). Evidence from Sertoli cell depleted rats indicate that spermatid number in adults depends on number of Sertoli cells produced during perinatal development. Endocrinology *122*, 787-794.

11. Roosen-Rung, E. C. (1973). Germinal cell loss in normal metazoan spermatogenesis. Journal of Reproduction and Fertility *35*(2), 339-348.

12. Segatell, T. M., Franca, L. R., Pinheiro, P. F. F., Alemida, C. C. D., Martinez, M., & Martinez, F. E. (2004). Spermatogenic cycle length and spermatogenic efficiency in the Gerbill (*Meriones unguiculatus*). Journal of Andrology *25*(6), 210-222.

13. Sinowatz, F. & Amselgruber, W. (1986). Postnatal development of bovine sertoli cells. Anatomy and Embryology *174*(3), 413-423.

14. Wing, T. Y., & Christensen, K. (1982). Morphometric studies on rat seminiferous tubules. The American Journal of Anatomy *165*(1), 13-25.