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Comparative histomorphometrical study on testis of indigenous Bull and Black Bengal Buck

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Abstract

Context: A comprehensive knowledge of normal histomorphology of testis is important for detecting testicular abnormalities.

Objectives: The present study was designed to study and compare the histomorphometrical features of testis of indigenous bulls (*Bos indicus*) and Black Bengal bucks (*Capra hircus*) at the age just after puberty.

Materials and Methods: Histomorphometry of the testis of indigenous bulls (n=5) and Black Bengal bucks (n=5) at the age just after puberty was studied and compared using routine Hematoxylin and Eosin (H&E) staining technique.

Results: The testis of both species was surrounded by visceral layer of tunica vaginalis (consisted of mesothelium and connective tissue) and tunica albugenia. The seminiferous tubules were tortuous, two ended loops and varying in appearance and the wall of tubules consisted of lamina propria, basement membrane and a lining of complex stratified epithelium consisted of Sertoli cells and spermatogenic cells. The sertoli cells are irregularly columnar cells, extended from basal lamina to lumen of tubules and the spermatogenic cells situated between the Sertoli cells in an orderly manner with four to eight layers (in bull) or four to six layers (in buck) occupying the space between the basal lamina and the lumen of the tubules. There was presence of both spermatid and spermatozoa in the lumen of some seminiferous tubules of testes of both species. The interstitial tissues located between the seminiferous tubules, consisted of connective tissue network, blood and lymph vessels with stromal cells. The stromal cells including the Leydig cells were present as single or groups within intertubular spaces. The thickness of tunica albuginea, cross sectional length and breadth of the seminiferous tubules and the number of stromal cells were significantly ($p<0.01$) more in the testis of bulls than bucks. There was a significant ($p<0.01$) correlation ($r=1.0$) between the length and breadth of seminiferous tubules of testis of both species of animals. In between left and right testes, the thickness of tunica albuginea and cross sectional length and breadth of the seminiferous tubules were higher in the left testis but the number of stromal cells was higher in right testis in both indigenous bulls and Black Bengal bucks.

Conclusions: The histological architecture of bull and buck was same with some histomorphometrical differences. The thickness of tunica albuginea, cross sectional length and breadth of the seminiferous tubules and the number of stromal cells were significantly higher in the testis of bulls than bucks.

Key words: Histomorphology, Puberty, Seminiferous Tubules, Spermatogenic cells, Stromal cells

Introduction

Livestock sector contributes significantly in agro-economy of Bangladesh. It generates about 13% of total foreign exchange earnings and provides fulltime employment to about 25% and partial employment to about 50% of the rural population (Rahman *et al.,* 2014). The total ruminant livestock population of Bangladesh is composed of 22.97 million cattle, 20.40 million goats, 1.30 million buffaloes and 2.87 million sheep (Huda *et al.,* 2014). The indigenous bulls and Black Bengal bucks are the major components of livestock of Bangladesh. They significantly contribute to the GDP and rank first and second in terms of meat, milk and skin production respectively in Bangladesh (Husain *et al*., 1998).

Most of the cattle population of Bangladesh is indigenous type (Islam *et al.,* 2010) though the reproductive performance of the indigenous bull is very poor in comparison to those of different pure breeds. Various efforts have been made in the recent past in Bangladesh to improve the semen quality of indigenous bull through cross breeding or by upgrading programme with different pure breeds (Rultedge, 1997). Black Bengal goat is the only recognized breed amongst the domestic species available throughout Bangladesh. Black Bengal buck has a notable goat genetic resource (Gofur, 2015). It produces excellent quality flavored tender and delicious meat (cheavon) and skin of extra ordinary quality for which there is huge demand all over the world (Husain, 1993; Islam *et al*., 1991).

Male reproduction is one of the important aspects in livestock that greatly influence the economy of any livestock production and business. Acute shortage of genetically superior bulls and bucks throughout the country is one of the major constraints of livestock production in Bangladesh (Husain, 2004). Superior bull and buck selection seems to be very important and alternative approach to boast up the production potential (Herrod, 2012). Therefore, during selection of breeding bull and buck special attention should be given on age, soundness of the sexual organ and quality of ejaculated semen. Brito *et al*. (2002) reported that age and bull genetics affect the characteristics of scrotum, testes, testicular vascular cone, sperm production and semen quality.

Testis is the main organ of male reproductive system and the main source of androgen (testosterone) that is responsible for male sexuality and secondary male sex characteristics (Eurell and Frappier, 2006; Hafez, 2000, Gofur *et al.,* 2014). The economic advancement of early puberty in male is important and the age when a bull/buck reaches puberty has a direct effect on the age when it can selected for progeny testing (Amann, 1983). Testicular architecture has been disorganized in various diseases involving the gonads such as hypogonadotropic eunuchoidism, Sertoli-cells-only syndrome

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(Gat *et al.,* 2010). Quantitative testicular histology has been used to determine daily sperm production in animals (Chenoweth and Lorton, 2014).

Knowledge of testicular development and architecture of indigenous bulls and Black Bengal bucks is important for the anatomist, pathologist, theriogenologist and livestock personnel of Bangladesh. As there is limited literature regarding this issue, the present study was designed to study and compare the histomorphometrical features of testis of indigenous bulls and Black Bengal bucks.

Materials and Methods

Animals

The present study was designed for comparative histomorphometrical study on testes of indigenous bull and Black Bengal buck. The bulls (n=5) of 1 year 9 months to 2 years of age and bucks (n=5) of 7 months to 1.0 year of age just after puberty were used for this study. The age at puberty was considered for indigenous bull and Black Bengal buck in the present study according to Hafez (2000). The age was determined by dentition according to eruption chart of Rahman *et al.* (2004).

Histomorphological study

Immediately after slaughter, the testes of bulls and bucks were collected. Then the testes were cut into small pieces. Small pieces of testicular tissue which were free from pathological lesion were used in this study. The small pieces of testes were fixed in the "Bouin's fluid" (Gridley, 1960). After fixation, the selected samples were processed in the laboratory following standard histological method, and the paraffin sections were then cut at 6 µm thickness using sliding microtome (MIC 509, Euromex, Japan). After cutting, the sections were floated on luke-warm water in a floatation bath at 37°C for stretching, then the sections were attached on cleaned glass slides using egg albumin and dried on a hot plate of slide warmer boxes. The sections were then stained with routine Hematoxylin and Eosin stain (Gridley, 1960) for histomorphological study of the testes of indigenous bull and Black Bengal buck. After staining, the sections were dehydrated in ascending grades of alcohol, cleared in xylene and mounted with "DPX". The stained sections of testes were studied thoroughly under light microscope using 10 and 40 objectives. The thickness of tunica albuginea and histological sectional length and breadth of the seminiferous tubules were measured using calibrated scale by adjusting ocular grid and stage microscope. The stromal cells including the Leydig cells in the sections of both left and right testes of both species were counted in 20 fields using ocular micrometer at a magnification of 40 where stromal cells were diffusely distributed and their relative frequency per 0.1 mm^2 was calculated according to Weibel (1969).

Statistical analysis

The frequency of stromal cells, thickness of tunica albuginea and cross sectional lengths and breadths of seminiferous tubules were compared in between bull and buck as well as in between left and right testes of the same species by using student's *t*-test (Zar, 1996) and the correlation between the length and breadth of seminiferous tubules was measured by Pearson's correlation analysis (Bivariate) with the help of SPSS Statistics version 20.

Results and Discussion

A comprehensive knowledge of normal histomorphology of testis has clinical significance, particularly for detecting testicular abnormalities. The present study described the histomorphometrical features of testes of indigenous bulls (*Bos indicus*) and Black Bengal bucks (*Capra hircus*) at the age just after puberty and the differences between them.

Covering of the testis

The testis was covered with visceral layer of the tunica vaginalis (consisted of mesothelium and connective tissue layer that blended with the tunica albuginea) and tunica albuginea. This finding is similar to the findings of Bacha and Bacha (2012), Eurell and Frappier (2006), Johnson *et al.* (1970) and Copenhaver *et al.* (1978). The thickness of tunica albuginea varied in between bulls and bucks and even left and right testes of same species (Fig. 1). The thickness of tunica albuginea was significantly ($p<0.01$) higher in bulls than the bucks. In bulls, the tunica albuginea was significantly ($p<0.05$) thicker in left testis than that of right testis but in bucks, the tunica albuginea of left testis was thicker than the right but the difference was not significant (Table 1). The measurement of thickness of tunica albuginea of bull was slightly higher than the observation of Copenhaver *et al.* (1978) in man and it was 500 µm but thickness in buck was somewhat near to this observation. It may be due to species variation. Reports regarding the variation of thickness of tunica albuginea of testes in different animals were not found in available literature. This result comments that the variation in thickness of tunica albuginea differs due to species difference.

Septula testis

The septula testis (trabeculae) were inconspicuous connective tissue strands divided the testicular parenchyma into a varying number of pyramidal and cone-shaped testicular lobules, each contained one to four convoluted seminiferous tubules in both bulls and bucks. These findings are similar to the observations of Eurell and Frappier (2006), Copenhaver *et al.* (1978), Stiles (1956).

Seminiferous tubules

The seminiferous tubules comprised the major part of testicular parenchyma. The tubules were tortuous two-ended loops, round and oblong in outline, varying in appearance due to the complex coiling of the tubules at different angles and levels. These findings are similar to the observations of Eurell and Frappier (2006) and Hafez (2000). The wall of seminiferous tubules consisted of lamina propria, basement membrane and a lining of complex stratified epithelium which consisted of Sertoli cells and spermatogenic cells. This finding is in agreement to the observations of Ham (1979) and Copenhaver *et al.* (1978).

The average length and breadth of the sections of seminiferous tubules of left and right testes of bulls and bucks were presented in Table 2. The cross sectional length and breadth of

seminiferous tubules was significantly ($p<0.01$) higher in bulls than the bucks. The cross sectional length and breadth of seminiferous tubules was significantly (bull) or insignificantly (buck) different between the right and left testis of same species (Table 2). The variation on length and breadth of cross section of seminiferous tubules might be due to testicular volume and species differences. The measurement of breadth of the sections of seminiferous tubules in the present study somewhat agrees with the finding of Jr. Machado *et al*. (2008) who reported the average diameter of seminiferous tubules was 215.49 µm, at 1.5 yrs of age in male goats. The sectional diameter of seminiferous tubules in the present study was to some extent similar with the results of other researchers (Bitto *et al*., 2008; Leal *et al*., 2004 and Setchell and Brooks, 1988). The present finding is somewhat below compared to Silva *et al.* (2015) as they found the mean diameter of the seminiferous tubules was 229.8 ± 1.22 µm in Nellore bulls in Brazil. The histomorphometric study of testis in rats was done by Kumar and Nagar (2014) and in mice by Viveka *et al.* (2015) and the mean diameter of the seminiferous tubules was 219.87 ± 4.44 µm in rats and 227.42 ± 7.25 µm in mice respectively. The correlation between the length and breadth of seminiferous tubules was also measured and found significant ($p<0.01$) correlation ($r=1.0$) between the length and breadth of seminiferous tubules of testis in both indigenous bull and Black Bengal buck.

Sertoli cell

The Sertoli cells were irregularly columnar cells that extended from the basal lamina to the lumen of tubules. The cells were appeared as clean other than the nuclei in routine staining method in the tubules of both species. The nuclei were oval and pear shaped. The location of the nuclei varied in different Sertoli cells from the basal lamina to at a considerable distance from the basal lamina. These findings are similar to the observations of Copenhaver *et al.* (1978), Hafez (2000), Eurell and Frappier (2006) and Bacha and Bacha (2012).

Spermatogenic cells

The spermatogenic cells were situated between the Sertoli cells in an orderly manner with four to eight layers (in bull) or four to six layers (in buck) occupying the space between the basal lamina and the lumen of seminiferous tubules (Fig. 2). This finding is similar to the observations of Copenhaver *et al.* (1978). The primitive germ cells or spermatogonia, from which all of the spermatozoa were ultimately derived, were located directly inside the basement membrane. They were spherical or cuboidal in shape and had spherical nuclei. The primary spermatocytes were next to the spermatogonia on their inner side. They were large cells and their nuclei were round or spherical. They had the biggest nuclei than the nuclei of other spermatogenic cells. The secondary spermatocytes were internal to the primary spermatocytes and smaller than the primary spermatocytes. The spermatids adjoined the lumen of the tubules and they were easily recognized by their small size and location and the spermatozoa were found in the lumen of the tubules. These findings are alike to the observations of Hafez (2000) and Copenhaver *et al.* (1978). There were presence of spermatids instead of spermatozoa and sometimes presence of both spermatids and spermatozoa in the lumen of some seminiferous tubules of testes of bulls and bucks (Fig. 3). This may be a sign of defective spermatogenesis and for this reason we sometime found both the spermatid and spermatozoa in the semen of bulls and bucks which reduces the fertility value of semen and may be a cause of failure of conception of cow. The information about the presence or absence of spermatid in the lumen of seminiferous tubules of testes was not available in literature.

Table 1. Thickness of tunica albuginea and frequency of stromal cells in left and right testes of indigenous bulls and Black Bengal bucks (mean ± SE).

Parameters	Thickness of tunica albuginea (µm)		Frequency of stromal cells	
	Left	Right	Left	Right
Bull (1 yr 9 months -2 yr)	950.35±1 63.52^{**a}	800.17±1 31.40^{**b}	67.00± 3.05^{**b}	82.67± 3.93^{**a}
Buck (6 months-1 yr)	326.61± 18.15^{a}	306.58± 14.08^{a}	58.50± 3.93^{a}	66.25± 2.72^{a}

In column, **$p<0.01$ and in row, same latter means non-significant and different letter means significant ($p<0.05$)

Interstitial tissues

The interstitial tissues lay between the seminiferous tubules (intertubular spaces) and consisted of loose connective tissue network, blood and lymph vessels with stromal cells including the Leydig cells (Fig. 4). The interstitial or stromal cells were polymorphous with spherical nuclei and occured as a single or in groups. These findings are similar to the previous observations by Eurell and Frappier (2006), Cole and Cupps (1977), Copenhaver *et al.* (1978), Malhi *et al.* (1999) and Goyal and Dhingra (1973).

The frequency of stromal cells including Leydig cells varied in the testes of bulls and bucks and even in between left and right testes of same animal. The number of stromal cells was significantly ($p<0.01$) higher in bulls than the bucks and may be due to difference in species and testicular volume. In bulls, the frequency of stromal cells was significantly ($p<0.05$) more in right testis than the left and in bucks, frequency pattern was same but the difference was insignificant (Table 1).

Table 2. Comparative measurements of the cross sectional length and breadth of seminiferous tubules of left and right testes of indigenous bulls and Black Bengal bucks (mean ± SE).

Parameters	Sectional length of seminiferous tubules (µm)		Sectional breadth of seminiferous tubules (µm)	
	Left	Right	Left	Right
Bull	634.81±2 2.72^{**a}	536.45±1 8.29^{**b}	191.69± 7.04^{**a}	161.87± 11.53^{b}
Buck	487.55±2 3.73^{a}	458.84±2 5.42^{a}	158.54± 13.13^{a}	146.17± 4.54^{a}

In column, **$p<0.01$ and in row, same latter means non-significant and different letter means significant ($p<0.05$)

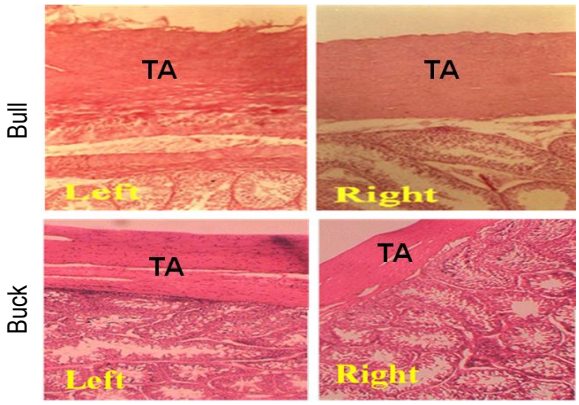

Figure 1. Histological section of testis of bull and buck; TA-Tunica albuginea

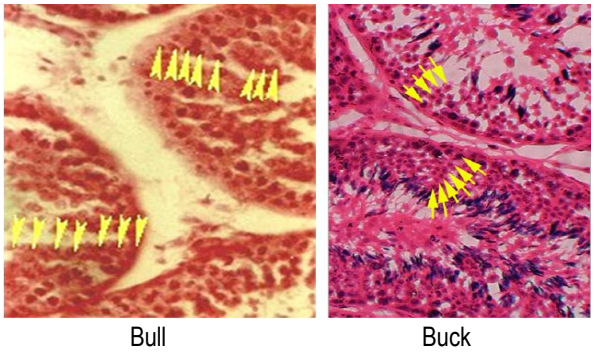

Figure 2. Histological sections of testis showing stratification of spermatogenic cells (arrow heads) in seminiferous tubules of bull and buck

The average number of stromal cell in 0.1 mm^2 areas obtained in the present study ranged from 67 ± 3.05 to 82.67 ± 3.93 and 58.50 ± 3.93 to 66.25 ± 2.72 in bulls and bucks respectively which are similar to some extend with the observation of former investigators (Leal *et al.,* 2004; Delgadillo *et al.,* 1995). There is no available literature about species basis difference on frequency of stromal cell of testis. However, Goyal and Dhingra (1973) studied on the postnatal histology of the testis in buffalo from birth to one year and concluded that the number of Leydig cell increase with age.

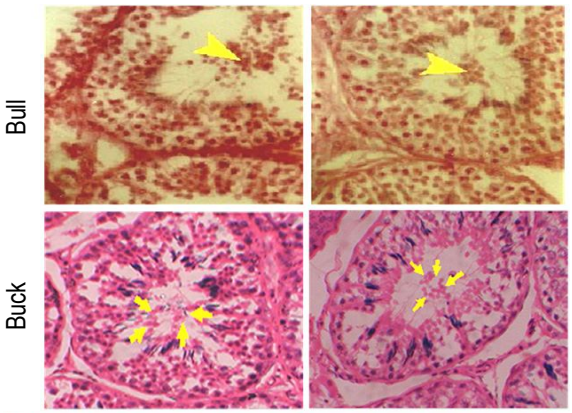

Figure 3. Histological sections of testis showing presence of spermatids (arrow heads) in the lumen of some seminiferous tubules of bull and buck

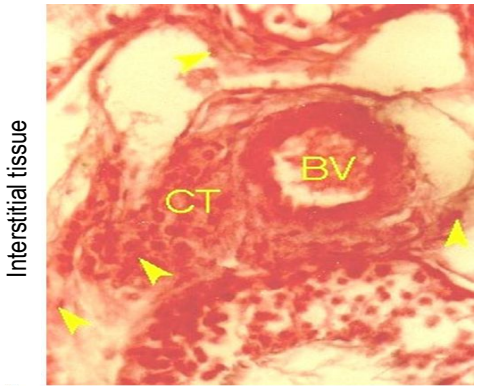

Figure 4. Histological section of testis showing interstitial tissue consisted of connective tissue fibers (CT) and stromal cells (arrow head), BV- Blood vessel

Conclusions

The histological architecture of bull and buck at the age just after puberty was same with some histomorphometrical differences. The thickness of tunica albuginea, cross sectional length and breadth of the seminiferous tubules and the number of stromal cells were significantly higher in the testis of bulls than bucks. There was a significant ($p<0.01$) correlation ($r=1.0$) between the length and breadth of seminiferous tubules of testis of both species of animals. In between left and right testes, the thickness of tunica albuginea and cross sectional length and breadth of the seminiferous tubules were higher in the left testis but the number of stromal cells was higher in right testis in both indigenous bulls and Black Bengal bucks.

Author's contribution

Authors have no conflict of interest to report.

References

Amann RP. Endocrine changes associated with onset of spermatogenesis in Holstein bulls. J. Dairy Sci. 1983; 66: 2606-2622.

Bacha WJ and Bacha LM. Color Atlas of Veterinary Histology. 3rd edn., Lea & Febiger. Philadelphia, USA. 2012. pp. 225-226.

Bitto II, Egbunike GN and Akusu MO. Seasonal variations in the histometric characteristics of the reproductive organs of pubertal West African Dwarf bucks in the native tropical environment. Int. J. Morpho. 2008; 26(2): 397-401.

Brito LFC, Silva AEDF, Rodrigues LH, Vieira FV, Deragon LAG and Kastelic JP. Effect of age and genetic group on characteristics of the scrotum, testes and testicular vascular cones, and on semen production and semen quality in AI bulls in Brazil. Theriogenol. 2002; 58: 1175-1186.

Chenoweth PJ and Lorton S. Animal Andrology: Theories and Applications. 1st edn., Cabi publications. USA. 2014. p. 21-25.

Cole HH and Cupps PT. Reproduction in Domestic Animals. 4th edn., Academic Press, New York, USA. 1977. pp. 221- 234.

Copenhaver WM, Kelly DE and Wood RL. Bailey's Text Book of Histology. 17th edn., The Williams and Wilkins Company. Philadelphia, USA. 1978. pp. 611-643.

Delgadillo JA, Hochereau-de MT, Daveau A and Chemineau P. Effect of short photoperiodic cycles on male genital tract and testicular parameters in male goats (*Capra hircus*). Reprod. Nutr. Dev. 1995; 35: 549-558.

Eurell JA and Frappier BL. Dellmann's Textbook of Veterinary Histology. 6th edn., Williams and Wilkins, A Waverly Company, Philadelphia, USA. 2006. pp. 233-243.

Gat Y, Gornish M, Perlow A, Chakraborty J, Levinger U, Ben-Shlomo I and Pasqualotto F. Azoospermia and Sertoli-cell-only syndrome: hypoxia in the sperm production site due to impairment in venous drainage of male reproductive system. Andrologia. 2010; 42(5): 314-321.

Gofur MR, Hossain KMM, Khaton R and Hasan MR. Effect of testosterone on physio-biochemical parameters and male accessory sex glands of black Bengal goat. Int. J. Emerg. Tech. Adv. Eng. 2014; 4(9): 456-465.

Gofur MR. Anatomy and histomorphometry of accessory reproductive glands of Black Bengal buck. European J. Anat. 2015; 19(2): 171-178.

Goyal HO and Dhingra LD. A study on the postnatal histology of the testis in buffalo (*Bubalus bubalis*) from birth to one year. Acta Anatomica. 1973; 84: 237-250.

Gridley MF. Manual of Histologic and Special Staining Technique. 2nd edn., McGraw-Hill Book Company. USA. 1960. pp. 28-31, 82-83.

Hafez RSE. Reproduction in Farm Animals. 7th edn., Lea and Febiger. Philadelphia, USA. 2000. pp. 3-12, 37-43.

Ham AW. Histology. 5th edn., JB Lippuncott Company. Philadelpia, New York, USA. 1979. pp. 273-289.

Herrod L. Genetic benefits in reproduction of *Bos indicus* (Brahman) under harsh condition. Nuffield Australia Project (No 1107) report. 2012; p.3.

Huda ASN, Mekhilef S and Ahsan A. Biomass energy in Bangladesh: Current status and prospects. Renew. Sustain. Energy Rev. 2014; 30: 504–517.

Husain SS, Amin MR and Islam ABMM. Goat production and its breeding strategy in Bangladesh. In: Proceedings of first National Workshop on Animal Breeding held in Bangladesh Agricultural University, Mymensingh on 26th November, 1998. pp: 17-36.

Husain SS. A study on the productive performance and genetic potentials of Black Bengal goats. Ph.D. thesis. 1993. Bangladesh Agricultural University, Mymensingh, Bangladesh.

Husain SS. Preservation of buck semen and their use in Artificial Insemination for rapid genetic improvement of rural goats' population. Project paper Bangladesh Agricultural University Research Systems, Mymensingh, Bangladesh. 2004.

Islam MM, Topader AH and Rob A. Comperative study on the cost benefit between indigenous and cross bred cows reared in rural area of Dinajpur district. Bangladesh J. Anim. Sci. 2010; 39(1&2): 191-196.

Islam MR, Saadullah M, Howlider MAR and Huq MA. Estimation of live weight and dressed carcass weight from the different body measurements of goats. Indian J. Anim. Sci. 1991; 61: 460-461.

Johnson AD, Gomes WR and Vandermark NL. The Testes. 5th edn., Vol. 1. Academic Press. New York, USA. 1970. pp. 282, 443-533.

Jr Machado ANN, Oliveira LS, Conde J, Menezes DJA, Assis Neto AC, Alves FR and Carvalho MAM. Morphometric and volumetric ratio of testicular compartments in goat according to scrotal configuration. Brazilan J. Morphol. Sci. 2008; 25(1-4): 35-108.

Kumar A and Nagar M. Histomorphometric study of testis in deltamethrin treated albino rats. Toxic. Reports. 2014; 1: 401-410.

Malhi PS, Roy KS and Pawar HS. Histomorphological studies on the postnatal development of seminiferous tubules in testis of Indian Murrah buffalo (*Bubalus bubalis*). Buffalo J. 1999; 15: 215-223.

Rahman MM, Khan MSU, Samad MA and Rahman MH. Practical Animal and Poultry Hygiene and Management. 1st edn., Brahmaputra Packaging. Mymensingh, Bangladesh. 2004. pp. 29 and 126.

Rahman S, Begum IA and Alam MJ. Livestock in Bangladesh: distribution, growth, performance and potential. Livestock Res. Rural Dev. 2014; Volume 26, Article no.173. http://www.lrrd.org/lrrd26/10/rahm26173.html

Rultedge JJ. Cattle breeding system enabled by in vitro embryo production. Embryo Transfer Newsletter. 1997; 15(1): 14-18.

Setchell BP and Brooks DE. Physiology of Reproduction. 2nd edn., Raven Press, New York. 1988. pp. 753-836.

Silva KM, Zart AL, Brum KB and Fernandes CES. Histopathological and histomorphometric testicular characteristics associated to reproductive condition in *Bos indicus* (Nellore) bulls. Med. Vet. 2015; 36(3, supplement 1): 1935-1944.

Stiles AK. Handbook of Histology. 4th edn., McGraw Hill Company. USA. 1956. pp. 179-188.

Viveka S, Udyavar A, Shetty B, Kuriakose S and Sudha MJ. Histomorphometric effects of gemcitabine on Swiss albino mice spermatogenesis. Adv. Biomed. Res. 2015; 4: 29.

Leal MC, Becker-Silva SC, Chiarini-Garcia H and França LR. Sertoli cell efficiency and daily sperm production in goats (*Capra hircus*). Anim. Reprod. Sci. 2004; 1(1): 122-128.

Weibel ER. 1969. Stereological principles for morphometry in electron microscopic cytology. Int. Rev. Cytol. 1969; 26: 235-302.

Zar JH. Biostatistical analysis. 3rd edn., Prentice- Hall, Upper Shaddle River, New Jersey. 1996. pp. 123-129.