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HISTOMORPHOLOGY AND HISTOCHEMISTRY OF TESTIS OF INDIGENOUS BULL (*BOS INDICUS*) OF BANGLADESH

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ABSTRACT

Histomorphological and histochemical features of testes were studied in six adult indigenous bulls (*Bos indicus*) of two different age groups, 1 year 9 months to 2 years of age (group A) and 2 years 3 months to 2 years 6 months of age (group B) during the period from September 2006 to April 2007 by using Hematoxylin and Eosin (H&E) stain, Verhoeff's stain, Van Gieson's stain and Periodic Acid-Schiff Reaction (PAS) stain. The testes were surrounded by visceral layer of tunica vaginalis (consisted of mesothelium and connective tissue) and tunica albugenia mainly composed of collagen fibers. The seminiferous tubules were tortuous, two ended loops and varying in appearance and the wall of tubules consisted of lamina propria, basement membrane supported by reticular fibers and a lining of complex stratified epithelium consisted of sertoli cells and spermatogenic cells. The sertoli cells are irregulary 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 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 bulls of both age groups. The spermatogonia, primary spermatocytes and secondary spermatocytes showed more staining affinity than the spermatid in routine staining technique. The basement membrane of tubules, spermatid and spermatozoa showed positive affinity whereas spermatogonia, primary spermatocytes and secondary spermatocytes showed negative affinity to PAS stain. The interstitial tissues located between the sminiferous tubules, consisted of connective tissue network, mainly composed of collagenous and reticular fibers; blood and lymph vessels with Leydig cells. The Leydig cells were present as single or groups within intertubular spaces. It was concluded that the thickness of tunica albuginea, the stratification of growing spermatogenic cells and cross sectional length and breadth of the seminiferous tubules of testes were higher in the bull of group B than group A and the number of Leydig cells were more in the testis of group A than group B and 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 Leydig cells was higher in right testis in both age groups.

Key words: Testis, seminiferous tubule, Leydig cell, indigenous bull

INTRODUCTION

The economic advancement of early puberty in male is important and the age when a bull reaches puberty has a direct effect on the age when it can selected for progeny testing (Amann, 1983). Testis is the main organ of male reproductive system and the testicular parenchyma is composed of seminiferous tubules from which spermatozoa are produced that maintain generation and Leydig cells that produce testosterone which is responsible for male sexuality and secondary male sex characteristics (Copenhaver *et al.*, 1978; Dellmann and Eurell, 1998; Hafez, 2000). Testicular architecture has been disorganized in various diseases involving the gonads such as hypogonadotropic eunuchoidism, Sertoli-cells-only syndrome (Heller and Nelson, 1948). In several cases of testicular failure, the problem may be quantitative only, with a reduction in the number of one or more of the different types of germ cells (Paulsen, 1968). Quantitative testicular histology has been used to determine daily sperm production in the boar (Kennelly and Foote, 1964) and short horn bull (Swienstra, 1966).

It is well known that the reproductive performance of the indigenous bull is very poor in comparison to those of different pure breeds. As most of the cattle population of Bangladesh is indigenous type and, therefore, 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. For the upgrading the production potentiality including expressing heterosis in our country is used semen from exotic dairy breeds of known genetics on the local zebu cattle (Rultedge, 1997). Though there have some research report on bovine testicular measurements (Coulter and Foote, 1979), biometry of bull testis (Gofur *et al.*, 2007), testicular development and establishment of spermatogenesis in Holstein bulls (Curtis and Amann, 1981), development of testis in Assam goat (Baishya *et al.*, 1987), but no comprehensive study on the histomorphological and histochemical features of testes in indigenous bull of Bangladesh has yet been undertaken. Therefore, the present study has been carried out to understand the histology and histochemistry of testis of indigenous bull which provides valuable information to the anatomist, pathologist and theriogenologist.

MATERIALS AND METHODS

The present research was carried out on testes (both left and right) of six indigenous bulls (*Bos indicus*) of two different age after onset of the puberty for histological and histochemical studies during the period from September 2006 to April 2007 at the Laboratory of the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Animals

Six adult indigenous bulls were selected in different local slaughter houses at Mymensingh district in Bangladesh for histomorphological and histochemical study of testes. The bulls were then divided into two groups (group A and group B) on the basis of their age. Group A included the bulls (n = 3) of 1 year 9 months to 2 years of age (bearing 2 permanent incisor teeth) and group B included the bulls (n = 3) of 2 years 3 months to 2 years 6 months of age (bearing 4 permanent incisor teeth). The age was determined by dentition according to eruption chart of Rahman *et al.* (2004).

Histological and histochemical study

Immediately after slaughter, the testes 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 4 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 and with special stains such as Van Gieson's stain, Verhoeff's Elastic stain (Mallory, 1942) and Periodic Acid-Schiff Reaction (PAS) stain (Gridley, 1960)) for histochemical study of the testes of indigenous bull. 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 Leydig cells in the sections of both left and right testes of both age groups were counted in 20 fields using ocular micrometer at a magnification of 40 where Leydig cells were diffusely distributed and their relative frequency per 0.1 mm^2 was calculated according to Weibel (1969).

The frequency of Leydig cells, thickness of tunica albuginea and cross sectional lengths and breadths of seminiferous tubules were compared in between two age groups as well as in between left and right testes of the same group by using student's *t*-test (Zar, 1996).

RESULTS AND DISCUSSION

Covering of the testis

The testes were covered with visceral layer of the tunica vaginalis (consisted of mesothelium and connective tissue layer that blended with the tunica albuginea) and tunica albuginea, mainly composed of collagen fibers. Elastic fibers were mainly found in the tunica intima of blood vessels of tunica vasculosa. This finding was similar to Bacha and Wood (1990), Dellmann and Eurell (1998), Johnson *et al.* (1970), Copenhaver *et al.* (1978) and Trautmann and Fiebiger (1952). The thickness of tunica albuginea varied in between two age groups and even left and right testes of same age group. The thickness of tunica albuginea of left and right testes of bulls of group A were 950.35 ±163.52 µm and 800.17 ±131.40 µm, respectively and of group B were 739.43±103.72 µm and 683.17±131.10 µm, respectively (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 0.5 mm (500 µm). It may be due to species variation. The findings of the present study concluded that the tunica albuginea was comparatively thicker in group A than group B and even in same age group, in between left and right testes; it was significantly ($P<0.05$) thicker in left testis than the right testis (Fig. 1). Reports with regards to the variation of thickness of tunica albuginea of testes of bulls of different age and different testes of same animal were not found in available literature. This result comments that the variation in thickness of tunica albuginea may be due to different activeness of testes. The thickness of tunica albuginea lessens with the increment of age of bull after puberty.

Table 1. Thickness of tunica albuginea and frequencies of Leydig cells in left and right testes of bull of two different age groups

Group (n = 3)	Thickness of turnica albuginea (µm) (Mean ± SE)		Frequencies of Leading cells Mean ± SE	
	Left	Right	Left	Right
Group A	950.35±163.52*	800.17±131.40	67±3.05	82.67±3.93*
Group B	739.43±103.72*	683.17±131.10	57.33±3.18	65±4.73*

$p < 0.05$.

Septula testis

The septula testis (trabeculae) were inconspicuous connective tissue strands, mainly composed of collagen fibers divided the testicular parenchyma into a varying number of pyramidal and cone-shaped testicular lobules, each contained one to four convoluted seminiferous tubules . These findings were similar to the observations of Dellmann and Eurell (1998), Copenhaver *et al.* (1978), Stiles (1956), Maximow and Bloom (1952).

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 were similar to the observations of Dellmann and Eurell (1998) and Hafez (2000). The wall of seminiferous tubules consisted of lamina propria, basement membrane supported by reticular fibers (Fig. 2) and a lining of complex stratified epithelium which consisted of Sertoli cells and spermatogenic cells (Fig. 3). This finding was similar to the observations of Ham (1979) and Copenhaver *et al.* (1978). In this investigation, an increase in tubular convolution associated with decreased intertubular space was observed in advancing age (Fig. 4a-b). This finding had a similar agreement with Baishya *et al.* (1987).

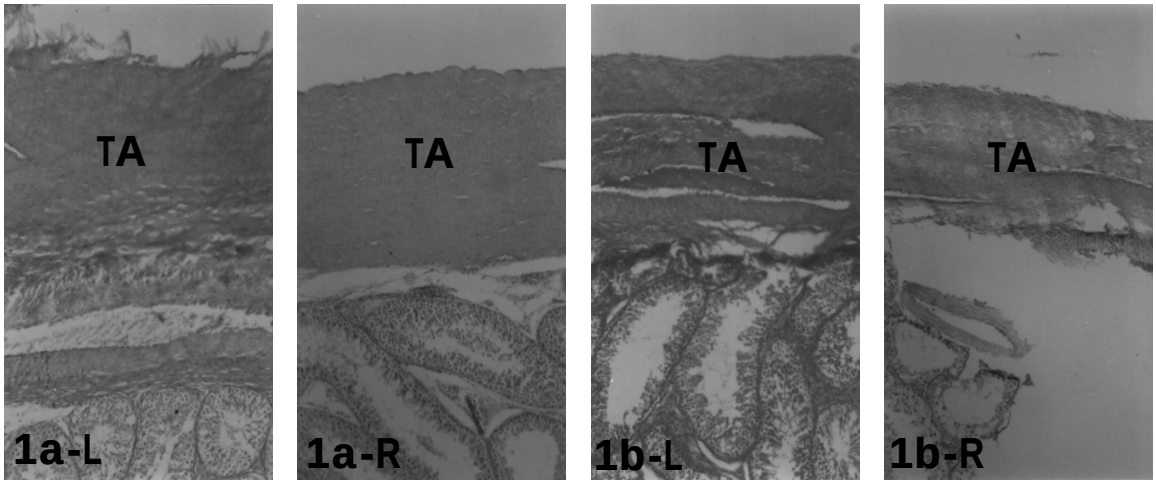

Fig. 1 (a-b). Histological section of the testis of indigenous bull showing thicker tunica albuginea in left testis than right one and in group A than group B; a = group A, b = group B, TA = Tunica Albuginea, L = Left, R = Right, Heamatoxylin and Eosin stain 82.5x.

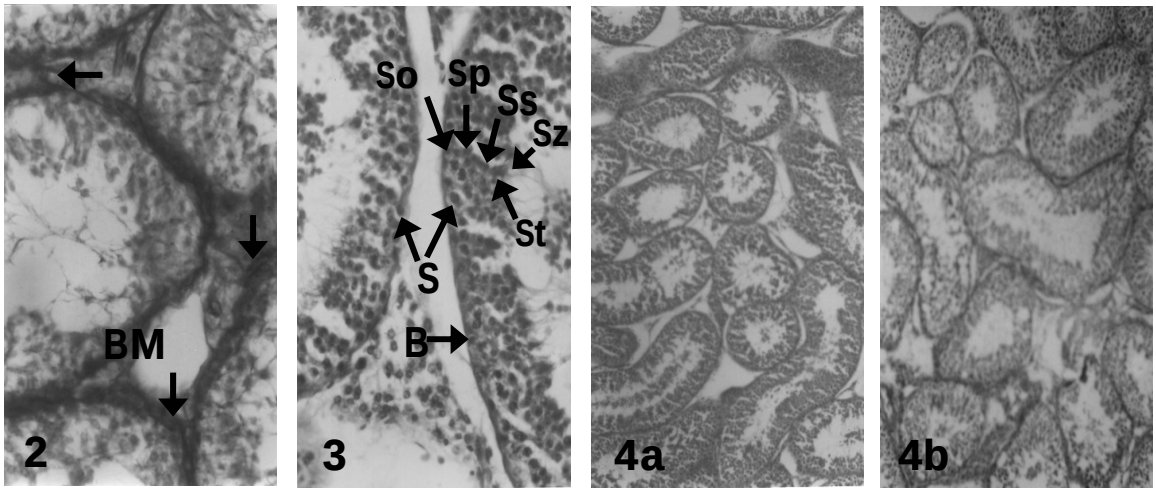

Fig. 2. Histochemistry of the testis of indigenous bull showing the basement membrane of seminiferous tubules surrounded by reticular fibers (showing by arrow heads); BM = Basement Membrane, Periodic Acid-Schiff Reaction (PAS) stain 330 x.

Fig. 3. Routine histological section of seminiferous tubules of testis of indigenous bull; B = Basement membrane, S = Sertoli cells, So = Spermatogonia, Sp = Primary spermatocyte, Ss = Secondary spermatocyte, St = Spermatid, Sz = Spermatozoa, Heamatoxylin and Eosin stain 330x.

Fig. 4 (a-b): Intertubular spaces among the cross sections of seminiferous tubules; a = group A, b = group B Heamatoxylin and Eosin stain 82.5x.

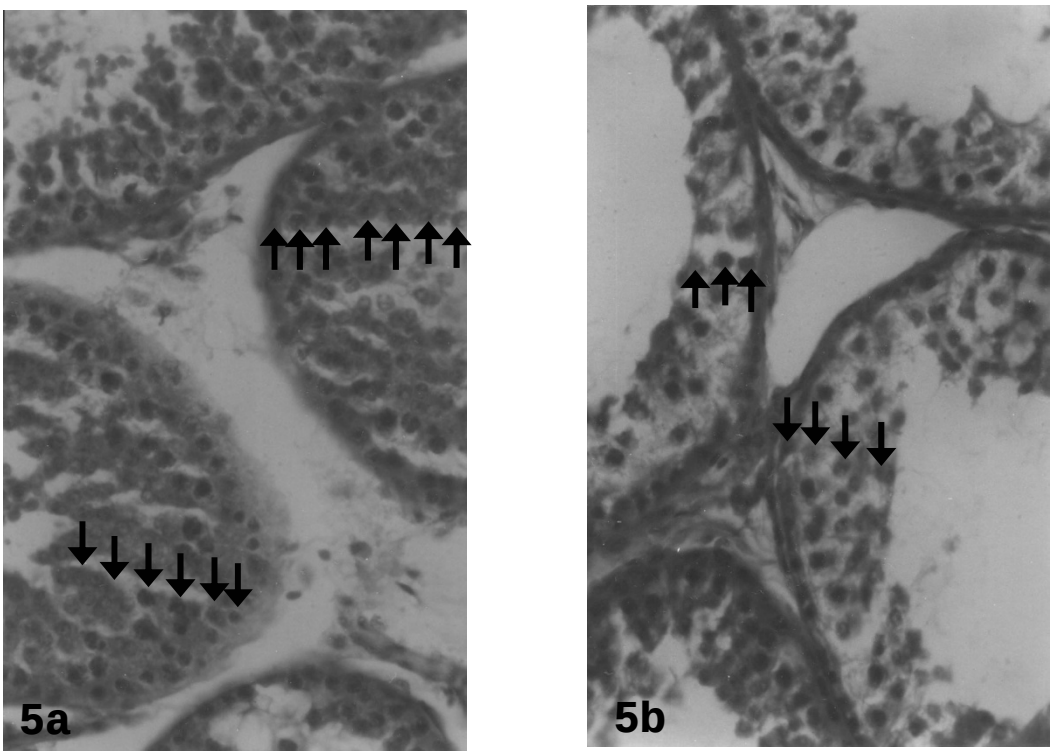

Fig. 5 (a - b). Stratification of growing spermatogenic cells showing by arrow; a = group A, b = group B, Heamatoxylin and Eosin stain 330x.

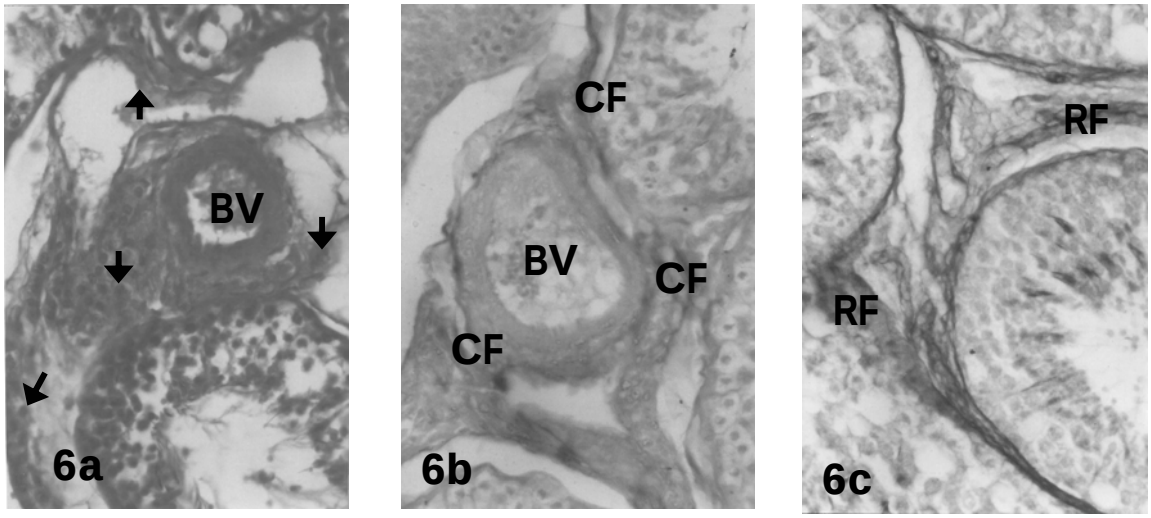

Fig. 6 (a - c). Interstitial tissue of testis of indigenous bull; Leydig cells showing by arrow; BV = Blood Vessels, CF = Collagen Fibers, RF = Reticular Fibers, Heamatoxylin and Eosin stain 330 x (6a), Verhoeff's and Van Gieson's stain 330 x (6b), Periodic Acid-Schiff Reaction (PAS) stain 330 x (6c).

The average lengths of the sections of seminiferous tubules of left and right testes of bulls of group A were 634.81±22.72 µm and 536.45±18.29 µm, respectively and breadths of them were 191.69±7.04 µm and 161.87±11.53 µm, respectively. The average lengths of the sections of seminiferous tubules of left and right testes of bulls of group B were 700.87±39.36 µm and 665.24±20.55 µm, respectively and breadths of them were 188.50 ±8.37 µm and 177.63±6.50 µm, respectively (Table 2). The length and breadth of the sections of seminiferous tubules of bulls of group B were higher than group A and in between left and right testes of both age groups, the lengths and breadths of the sections of seminiferous tubules of left testis were significantly ($P<0.05$) higher than the right testis. The length and breadth of cross section of seminiferous tubules incresed with the advancement of age. These findings had an similar agreement with Watson *et al.* (1956). This result suggests that the reproductive capacity is greater in group B than group A as reproductive capacity is greater in bulls with larger seminiferous tubules than smaller seminiferous tubles (Watson *et al.*, 1956).

Table 2. Comparative measurements of the cross sectional length and breadth of Seminiferous tubules of left and right testes of bull in between two different age groups.

Parameters	Group A (n = 3) (Mean ± SE)		Group B (n = 3) (Mean ± SE)	
	Left	Right	Left	Right
Length (in µm)	634.81±22.72*	536.45±18.29	700.87±39.36*	665.24±20.55
Breadth (in µm)	191.69±7.04*	161.87±11.53	188.50±8.37*	177.63±6.50

*$p < 0.05$.

Sertoli cells

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 age groups. 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 were similar to the observations of Copenhaver *et al*. (1978), Dellmann and Eurell (1998) and Hafez (2000).

Spermatogenic cells

The spermatogenic cells were situated between the Sertoli cells in an orderly manner with four to eight layers occupying the space between the basal lamina and the lumen of seminiferous tubules. This finding was 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 (Fig. 3). These findings were similar 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 bull of both age groups. This may be a sign of defective spermatogenesis and for this reason we may find the both spermatid and spermatozoa in the 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 of bulls of different age groups was not found in available literatures. The spermatogonia, primary and secondary spematocytes showed more staining affinity than the spermatids and spermatocytes in routine staining technique. The stratification of growing spermatocytes was more in the tubules of bull of group A (5-8 layers) than the group B (3-6 layers) (Fig. 5a-b).

On the other hand, spermatozoa were present in almost all tubules of group B but in group A, spermatozoa found only in a few tubules. This observation suggests that the bulls of older age contained more spermatozoa in the lumen of seminiferous tubules than the younger one. However the informations about the staining affinity of spermatogenic cells in routine staining technique, stratification of spermatogenic cells and presence or absence of spermatozoa in seminiferous tubules of bulls of different age groups were not found in available literature. But Johnston *et al.* (1982) reported that spermatocytes appeared in the seminiferous tubules by 7 to 9 months of age in bulls and as Malhi *et al.* (1999); spermatogenic process was completely established by 3 to 3.5 years of age in bulls. In PAS stain the basement membrane of seminiferous tubules, spermatids and spermatozoa showed positive affinity whereas the spermatogonia, primary and secondary spermatocytes and Sertoli cells showed negative affinity. These findings suggested that spermatid and spermatozoa contained more glycogen as they were more active and contained hyaluronidase. The information about the staining affinity of different components of seminiferous tubules in PAS stain was not found in available literature.

Interstitial tissues

The interstitial tissues lay between the seminiferous tubules (intertubular spaces) and consisted of loose connective tissue network, blood and lymph vessels, fibrocytes and interstitial or Leydig cells. The collagen fibers predominate over the reticular fibers in this network while elastic fibers are absent (Fig. 6a-c). The later, however, were demonstrable in tunica intima of blood vessels in this region. The interstitial cells were polymorphous with spherical nuclei and granular cytoplasm. They occurred as single or in groups. These findings were similar to the observations of Dellmann and Eurell (1998), Cole and Cupps (1977), Copenhaver *et al.* (1978), Malhi *et al.* (1999), Sudhakar and Sharma (1993), Singh and Bharadwaj (1978), Hernandez and Marquez (1977), Fawcett *et al.* (1973) and Goyal and Dhingra (1973). The number of Leydig cells varied in the testes of bulls of different age groups and even in between left and right testes of same animal. The number of Leydig cell in 0.1 mm^2 areas of left and right testes of group A was 67.00±3.05 and 82.67±3.93 respectively and of group B was 57.33±3.18 and 65.00±4.73 respectively (Table 1). The number of Leydig cells in group A was more than group B. In between the left and right testes of both age groups, the number of Leydig cells of right testis was significantly ($P<0.05$) more than the left testis. 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 in contrast we observed that the number of Leydig cell increase up to puberty and then decrease with the advancement of age associated with an increase connective tissue fibers in the intertubuler space.

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