Original Research

Histogenesis of Testis in Gaddi Sheep Foetii

Parul Shukla* and Rajesh Rajput

Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Chaudhary Sarwan Kumar Krishi Vishvavidyalaya – 176062, Palampur, Himachal Pradesh, INDIA

***Corresponding author:** shukla.p.vet@gmail.com

Rec. Date:	Mar 11, 2019 06:52
Accept Date:	Apr 20, 2019 16:53
DOI	10.5455/ijlr.20190311065250

Abstract

The histogenesis of the testis was studied in sixty eight Gaddi sheep foetii ranging from 36th day to 140th day (2 cm to 40 cm CRL) of gestation. The foetii were measured for their crown-rump length (CRL) in cm and were divided into four stages on the basis of their age viz. stage I (31-60 days), stage II (61-90 days), stage III (91-120 days) and stage IV (121- till term). In the indifferent gonad the cortex and medulla were differentiable by 37th day of gestation. The tunica albuginea and seminiferous tubules containing large cells, small cells and sertoli cells were observed on 46th day of gestation. The tunica albuginea was distinguishable in outer tunica fibrosa and inner tunica vasculosa on 50th day of gestation. The process of convolution started in the developing sex cords by 61st day of gestation and seen clearly on 137th day of gestation.

Key words: Gonad, Gaddi, Histogenesis, Sheep, Testis

How to cite: Shukla, P., & Rajput, R. (2019). Histogenesis of Testis in Gaddi Sheep Foetii. International Journal of Livestock Research, 9(6), 255-263. doi: 10.5455/ijlr.20190311065250

Introduction

Testis is a primary male sex organ performing both exocrine and endocrine functions. Any disturbance in the development and maturation can lead to low reproductive efficiency or even infertility in animals and may reflect as unrecognized defects in the histogenesis of male gonad during critical organizational periods (Kaur *et al.*, 2011). Gaddi sheep is one of the important livestock breed of northern temperate region of India. The morphogenesis of prenatal testis has been studied in goat (Baishya *et al.*, 1987 and Farooqui *et al.*, 2012) and buffalo (Kaur *et al.*, 2011) but scanty literature is available on the prenatal development of testis in Gaddi sheep, so the present work was taken up.

Materials and Methods

The present study was conducted on the testes of 68 Gaddi sheep foetii. The foetii were measured for their crown – rump length (CRL) in cm with the help of a graduated nylon tape (Harvey, 1959) and their

approximate age was calculated by using the following formula given by Gall *et al.* (1994) *i.e*. Y = 2.74 X + 30.15, where Y is the age of embryos in days and X is the CRL in cm. Thereafter, the foetii were divided into four stages on the basis of their age *viz.* stage I (31-60 days), stage II (61-90 days), stage III (91-120 days) and stage IV (121- till term).

The right and left testes collected were fixed in 10% neutral buffered formalin. After required fixation period, the tissues were processed with alcohol-benzene schedule for paraffin technique. Sections cut at 5-6 µm thickness were stained with haematoxylin and Eosin method for general histomorphology, Verhoeff's Elastica stain for elastic and collagen fibres, Weigert's method for elastic fibres (Luna, 1968); Gomori's method for reticular fibres (Mallory, 1942) and Masson's Trichrome method for connective tissue (Lillie, 1948).

Results and Discussion

The outer lining of indifferent gonad was found to be simple squamous type at 37th day (CRL 2.5 cm) of gestation. No sex cords were observed in the gonads at this age. These findings corroborated well with the observations of Abd – Elmaksoud (2005) and Kaur (2006) in bovine fetuses. Beneath the epithelium, a distinct layer of mesenchymal cells was identified which may develop as future testicular tunica albuginea at 46 days (CRL 5.8 cm) of gestation (Fig. 1).

Germinal Epithelium

The simple squamous epithelium changed to low cuboidal type of epithelium except towards the mesothelium of the mesonephros (Fig. 1) on 46th day of gestation (stage II). The germinal epithelium became simple cuboidal and had similar characters on 63rd day of gestation (stage III) as in stage II of gestation. The findings were similar to that earlier reported by Farooqui *et al.* (2012) in goat.

Testicular Capsule

The tunica albuginea just beneath the germinal epithelium was composed of few layers of mesenchymal cells, fibroblasts and small capillaries (Fig. 1) on 46th day of gestation. Singh *et al.* (1979) and Farooqui *et al.* (2012) observed a distinct tunica albuginea on 47th day and 44th day of gestation in goat foetii, respectively.

The tunica albuginea was distinguishable in outer thicker tunica fibrosa and inner thin tunica vasculosa (Fig. 2) on 50th day of gestation. Kaur (2006) reported that the capsule differentiated into outer fibrous layer (tunica fibrosa) and inner vascular layer (tunica vasculosa) in buffalo foetii at 65 days of gestation.

DOI 10.5455/ijlr.20190311065250

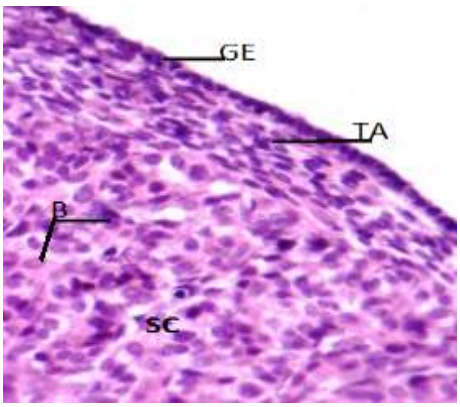

Fig. 1: Crosssection of the fetal testis showing germinal epithelium (GE), tunica albuginea (TA), small cells (B) and sex cords (SC). CRL – 5.8 cm. H & E x 400.

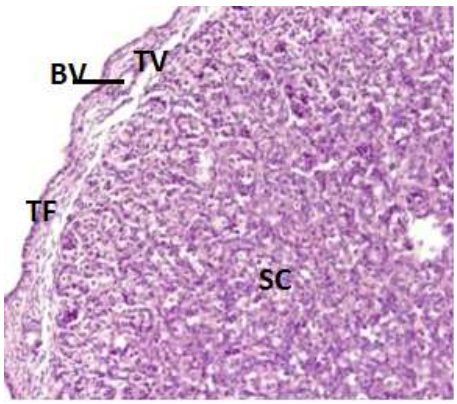

Fig. 2: Crosssection of the fetal testis showing tunica fibrosa (TF) and tunica vasculosa (TV) with large blood vessels (BV). Sex cords (SC) also visible. CRL – 7 cm. H & E x 100.

The connective tissue trabeculae invaginated into the testicular parenchyma dividing it into lobules which contained developing sex cords on 60th day of gestation (Fig. 3). The two layers of the capsule become more demarcable on 70th day of gestation (stage II). The outer layer of the capsule became more fibrous on 95th day of gestation (stage III). The capsule became more folded, compact and compressed in configuration. The number of blood vessels increased. In stage III of gestation the collagen (Fig. 4) and reticular fibers were well differentiated in the capsule. The fibrous layer of the capsule was more folded and compactly arranged on stage IV of gestation (121 days to term) than the previous stage. There was further increase in the vascularity at this stage.

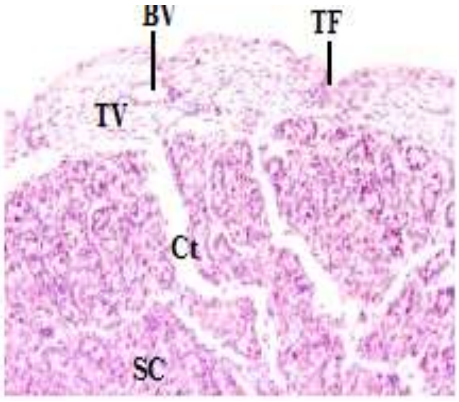

Fig. 3: Crosssection of the fetal testis showing tunica fibrosa (TF), tunica vasculosa (TV) containing blood vessel (BV) and connective tissue trabeculae (Ct) dividing the testicular parenchyma into lobules which contain developing sex cords (SC). CRL - 11.2 cm. H & E x 100.

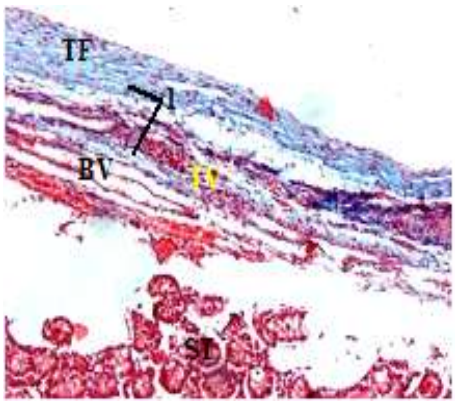

Fig. 4: Crosssection of the fetal testis showing collagen fibres (1) blood vessels (BV), tunica fibrosa (TF) and tunica vasculosa (TV). Seminiferous tubules (ST) present in the testicular parenchyma. CRL – 25 cm. Masson's trichrome method x 100.

DOI 10.5455/ijlr.20190311065250

The trabeculae were seen more clearly on 137th day (CRL 38.9 cm) of gestation. These trabeculae contained large amount of collagen and reticular fibres. Similar observations were made by Abd-Elmaksoud (2005) and Kaur (2006) in bovine and buffalo foetal testis respectively.

Seminiferous Tubules

The seminiferous tubules were first observed on 46th day of gestation (stage I) at the gonadal periphery (Fig. 1). Singh *et al.* (1979) and Farooqui *et al.* (2012) observed the process of formation of sex cords at 47th and 44th day of gestation in goat foetal testis, respectively. The developing sex cords contained small mesenchymal and large cells arranged in cords (Fig. 5). The presence of these cells in the sex cords had also been reported earlier in foetal testis of goat (Singh *et al.*, 1979 and Farooqui *et al.*, 2012) and cattle (Santamarina and Reece, 1957). The sex cords lacked the clear lumina and were filled with an irregularly oriented eosinophilic mass (Fig. 5). Several investigators used the term sex cords for seminiferous tubules until these were luminated (Goyal & Dhingra, 1973 and Chandra Pal, 1976). The testicular parenchyma was subdivided into two zones at 47th day of gestation. The outer zone contained oval to slightly elongated tubules whereas the inner zone had longitudinal tubules (Fig. 6). In contrast Kaur (2006) reported longitudinal tubules in the outer zone and rounded tubules in the inner zone of developing seminiferous tubules in the initial stages of first trimester of pregnancy in buffalo foetii.

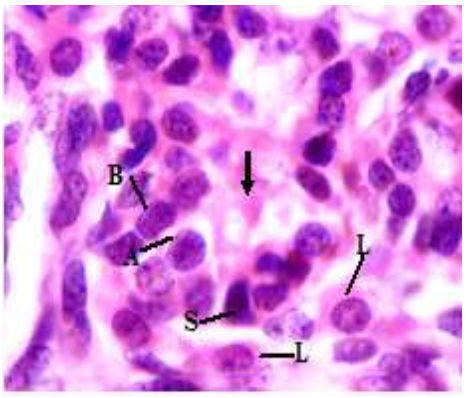

Fig. 5: Cross section of the fetal testis showing large cell (A), small cell (B), sertoli cell (S), leydig cell (L) and intertubular matrix (arrow). CRL 5.8 cm. H & E x 200.

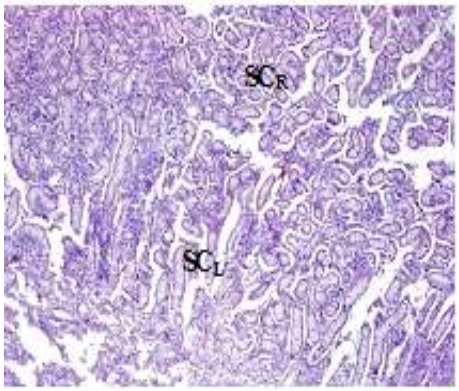

Fig. 6: Crosssection of the fetal testis showing oval to elongated sex cords (SC_R) in outer zone and longitudinal sex cords (SC_L) in inner zone of testicular parenchyma.CRL 6.3 cm. H & E x 40.

The presence of slight concavity in the sex cords indicated the beginning of convolution in sex cords (Fig. 3) on 61st day of gestation (stage II). With the advancement of gestation the process of convolution progressed in the sex cords located towards the centre of the parenchyma. The collagen fibers were present surrounding the developing seminiferous tubules. The degree of tortuousness was higher as compared to the previous stage at 99th day (stage III) of gestation and due to increased degree of convolution the

 DOI 10.5455/ijlr.20190311065250

seminiferous tubules appeared "C" shaped at 136th day of gestation (stage IV). Abundant reticular and collagen fibers were present at the periphery of the tubules in stage IV. The seminiferous tubules surrounded by a distinct basement membrane and a layer of peritubular cells were observed at 47th day of gestation. These cells were elongated in shape with a rounded nucleus. These cells are responsible for maintaining the structural integrity of seminiferous tubules.

Gonocytes

The seminiferous tubules comprised of large and small cells in initial stages. The sertoli cells were present in between large and small cells.

Large Cells

The large cells were first observed at 46th day of gestation (stage I) (Fig. 5).These cells were spherical or oblong in shape with distinct cell boundaries. The cytoplasm was vacuolated and weakly eosinophilic (Fig. 7) as reported earlier by Farooqui *et al.* (2012) in goat. In stage I, large cells were located at the periphery close to the basement membrane and few cells were present towards the centre of the developing seminiferous tubules. That might had been an indication of the movement of the cells towards the future lumen of cords as observed by Farooqui *et al.* (2012) in goats. In contrast Singh *et al.* (1979) and Baishya *et al.* (1987) in goats and Baishya and Vyas (1990) in Surti buffalo reported the presence of large cells in the centre of the tubules. The large cells revealed hypertrophic and degenerative changes with more vacuolations as compared to earlier stages (Fig. 8) on 86th day of gestation. The large cells decreased in number at stage III of gestation. Cytoplasm of most of the degenerative cells showed large sized vacuoles.

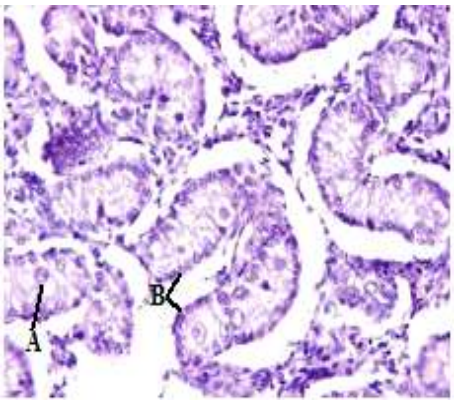

Fig. 7: Cross section of the foetal testis showing large cell (A) with clear cytoplasm and small cell (B). CRL 6.3 cm. H & E x 200.

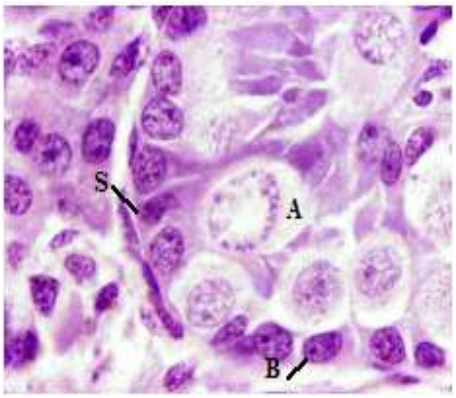

Fig. 8: Cross section of the foetal testis showing large cell (A) with more vacuolations and degenerative changes, small cell (B) and sertoli cell (S). CRL 20.5 cm. H & E x 1000.

The large cell population was further decreased from 122nd day of gestation (stage IV). Such cells contained centrally placed nucleus with densely staining chromatin granules (Fig. 9). The degenerative changes,

DOI 10.5455/ijlr.20190311065250

observed in large cells in our study were in agreement with that stated by Singh *et al.* (1979) and Farooqui *et al.* (2012) in goat and Baishya and Vyas (1990) in buffalo foetal testis. These authors opined that the primordial germ cells undergo the process of degeneration with advancement of gestation.

Small Cells

These cells were first observed on 46th day of gestation (stage I) in (Fig. 5). Farooqui *et al.* (2012) and Singh *et al.* (1979) observed these cells in goat foetal testis on 44th and 48th day of gestation, respectively. These cells were spherical oval or irregular in shape and were located peripherally close to the basement membrane of developing sex cords (Fig. 7). The cytoplasm of such cells was relatively more eosinophilic than that of the large cells. Spherical or oval nuclei of these cells contained evenly distributed, finely granular chromatin (Fig. 8). These cytological characters were in agreement with Farooqui *et al.* (2012) in goat. The small cells had similar nuclear characters on 69th day (stage II) of gestation as observed in stage I. The nucleolus showed intense staining with eosin (Fig. 8) on 86th day of gestation. There was further increase in number of small cells on 95th day of gestation than the previous stages. They formed a layer along the basement membrane. These cells had granulated eosinophilic cytoplasm and nuclear chromatin exhibited clump formation (Fig. 9).The mitotic figures were present in some of the small cells on 136th day (stage IV) of gestation. The cytoplasm of these cells became less eosinophilic than that of the previous stages.

Sertoli Cells

The sertoli cells were first observed in Gaddi sheep foetal testis on 46th day of gestation (stage I) located among small cells of the sex cords (Fig. 5). Farooqui *et al.* (2012) observed the sertoli cells on 56th day of gestation in goat foetal testis. They were roughly pyramidal in shape with indistinct cell boundaries. The cells showed cytoplasmic extensions from the supra nuclear zone. Farooqui *et al.* (2012) observed such extensions on 70th day of gestation in goat. The cells had pyramidal shaped nuclei on 61st day of gestation (stage II) and some of the cells had elongated nuclei and showed cytoplasmic extensions. At 86th day most of the cells had pyramidal shaped nuclei and only few cells had elongated nuclei (Fig. 8). The sertoli cells were in close contact with the basement membrane of sex cords on 95th day of gestation (stage III). The nuclei of these cells were cylindrical or pyramidal in shape with one or 2 nucleoli. These cells had darkly stained eosinophilic cytoplasm in the supra nuclear zone from where the cytoplasmic extensions were going towards the future lumen (Fig. 9) on 121st day (stage IV) of gestation.

Interstitial Tissue

The interstitial tissue contained blood vessels, loose network of mesenchymal cells, collagen fibers, reticular fibers and interstitial endocrine cells.

DOI 10.5455/ijlr.20190311065250

Interstitial Endocrine Cells (Leydig cells)

At 46th day of gestation (stage I) few of the mesenchymal cells located in between the sex cords, started developing into future interstitial endocrine cells, characterized by their roughly polygonal shape and highly eosinophilic cytoplasm. The spherical nuclei of these cells were vesicular in nature and contained a distinct eccentrically placed nucleolus (Fig. 5). Singh *et al.* (1979) and Farooqui *et al.* (2012) observed interstitial endocrine cell differentiation at 44th and 47th day in goat foetal testis, respectively. The interstitium expanded due to differentiation of mesenchymal cells into leydig cells on 60th day of gestation and constituted the larger cell population of all the other components of interstitium at 139th day of gestation. This finding was in agreement with the observations of Orth (1993) and Kaur (2006) who found that the number of mesenchymal cells decreased with the appearance of leydig cells in pig and buffalo foetal testis respectively.

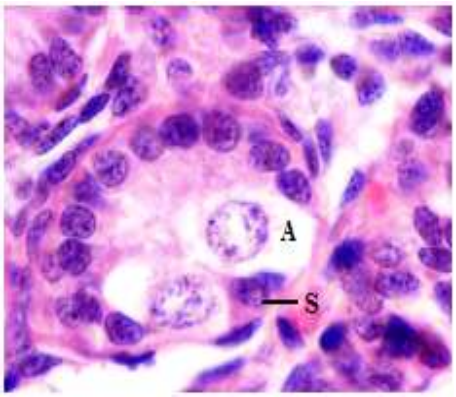

Fig.9. Cross section of the foetal testis showing sertoli cell (S) and large cell (A) with more vacuolations and degenerative changes. CRL 33.5 cm. H & E x 1000.

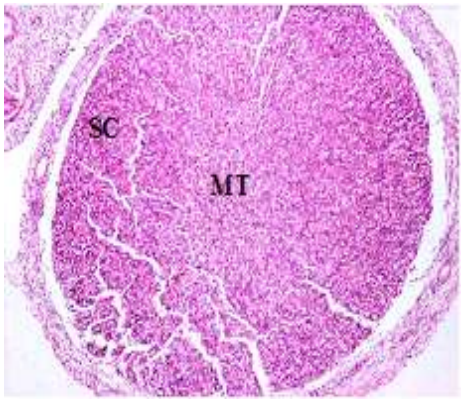

Fig. 10. Cross section of the foetal testis showing lightly stained region of mediastinum testis (MT) as compared to surrounding parenchyma which contained sex cords (SC). CRL 11.2 cm. H & E x 40.

Mediastinum Testis

The mediastinum testis was observed on 46th day of gestation (stage 1) as an area devoid of sex cords. Singh *et al.* (1979) and Farooqui *et al.* (2012) observed the anlagen of mediastinum testis at 48th and 44th day of gestation respectively in goat foetal testis. The connective tissue core of mediastinum testis increased further at 61st day of gestation (stage II) and at this age the area was poorly stained in H & E preparation as compared to surrounding parenchyma of the testis (Fig. 10). The area of mediastinum testis showed improved staining in later stages of gestation.

Rete Tubules

The rete tubules had clusters of cells in the area of mediastinum testis on 70th day of gestation. Vacuolation was observed in the central area of developing rete tubules marking the beginning of the lumen formation.

DOI 10.5455/ijlr.20190311065250

This observation was in agreement with the findings of Singh *et al.* (1979) and Farooqui *et al.* (2012) in goat. The lumen formation was further progressed on 88th day of gestation and the cells started to rearrange themselves around a small lumen (Fig. 11). The development changes on 108th day of gestation were similar to the previous stage with slight increase in the size of lumen. Most of the tubules were luminated and were surrounded by coarse reticular fibers which had branching and anastomosing pattern on 122nd day of gestation. Few of these rete tubules were observed making connections with tubuli recti (Fig. 11).

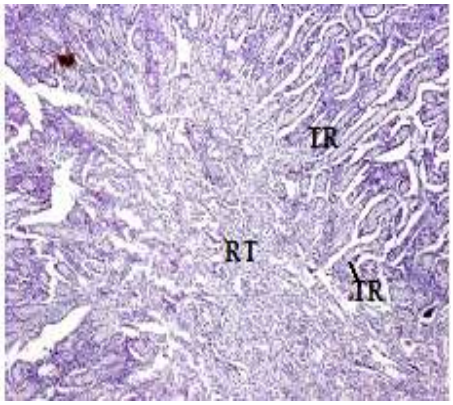

Fig. 11. Cross section of the foetal testis showing tubuli recti (TR) making connection with laminated rete tubules (RT). CRL 21.1 cm. H & E x 40.

Conclusion

The differentiation of cortex and medulla in the indifferent gonad was observed on 37th day of gestation. The germinal epithelium was of simple squamous type which became cuboidal type in later stages of gestation. The distinct tunica albuginea was observed on 46th day of gestation. The connective tissue trabeculae from the capsule divided the testicular parenchyma into lobules which contained developing sex cords on 60th day of gestation. The sex cords contained large cells and small cells with sertoli cells present in between these cells. The mediastinum testis was observed on 46th day of gestation as an area devoid of sex cords. The diameter of seminiferous tubules, large, small, sertoli and leydig cells increased with the progression of gestation.

Acknowledgement

The authors are thankful to DGCN college of Animal and Veterinary Sciences, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur for providing infrastructure and support for the conduct of this research work.

References

1. Abd – Elmaksoud, A. (2005). Morphological, glycohistochemical and immunohistochemical studies on the embryonic and adult bovine testis. Ph.D. thesis, Munich, Germany.

DOI 10.5455/ijlr.20190311065250

2. Baishya, G. and Vyas, K.N. (1990). Histomorphological development of foetal testis in the Surti buffalo. *Indian Journal of Animal Sciences* 60: 1425-30.
3. Baishya, G., Ahmed, S. and Bhattacharya, M. (1987). Development of testes in Assam goat (*Capra hircus*). *Indian Veterinary Journal 64*: 24-8.
4. Chandra Pal. (1976). Gross, histological and histochemical studies on male genital system of buffalo (Bubalus bubalis). Ph.D. thesis, submitted to Agra University, Agra.
5. Harvey, E.B. (1959). *Aging and foetal development. In:* Reproduction in Domestic Animals Cole, H.H. & Eupps, P.T. (Eds.). 1st ed., New York, Academic Press Inc. Vol. I. P 461 – 6.
6. Kaur, M., Bansal, N. and Uppal, V. (2011). Histogenesis of testicular parenchyma during prenatal life in buffalo. *International Journal of Morphology* 29: 1109 – 1114.
7. Kaur, M. (2006). Studies on histogenesis and organogenesis of the testis in buffalo (*Bubalis bubalis*) M.V.Sc. thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.
8. Lillie, R.D. (1948). In: Histopathological Technique. The Blakiston Company. Philadelphia. P 196.
9. Luna, L.G. (1968). *Manual of Histologic staining methods of Armed Forces Institute of Pathology.* 3rd ed. Mc Graw Hill Book Company New York. P 38 – 39, 87 – 88, 104 -105, 145, 158 -160, 163 – 164.
10. Farooqui, M.M., Chandrapal., Archana and Prakash, A. (2012). Histological and Histochemical Studies on the prenatal Development of Testis in Goat (*Capra hircus*). *International Journal of Morpholo*gy 30: 1408 – 1421.
11. Gall, C.F., Stier, C.H. and Fraham, K. (1994). Age estimation of goat fetus. *Small Ruminant Research* 14: 91 – 94.
12. Goyal, H.O. and Dhingra, L.D. (1973). Postnatal study on the histology and histochemistry of mediastinum testis in buffalo from birth to one year. *Acta Anatomica* 86: 65 – 70.
13. Mallory, F.B. (1942). In: *Pathological Technique*, WB Saunders Company. Philadelphia. p 170.
14. Orth, J.M. (1993). Cell biology of testicular development in the fetus and neonate. In: Desjardins C and Ewing LL (eds) Cell and Molecular Biology of the Testis. Oxford University Press, Oxford.
15. Singh, Y., Sharma, D.N. and Dhingra, L.D. (1979). Morphogenesis of the testis in goat. *Indian Journal of Animal Sciences* 49: 925 – 31.

DOI 10.5455/ijlr.20190311065250