CHAPTER 5

SUMMARY AND CONCLUSIONS

Gaddi sheep is one of the important livestock breed of northern temperate region of India. They are economically important for the local tribes of this region who obtain hair (wool) and meat by rearing these animals. It is an old breed with obscure origin presumed to have descended from ancient Asiatic sheep and brought to this area by Gaddi tribes. The animal is adapted to survive at cool and arid environment of high mountainous ranges with the altitude varying from 3500 to 5000 feet above mean sea level. Testis along with epididymis plays an essential role in normal reproduction of mammals. Testis along with production of sperms is also responsible for the secretion of endrogenic hormones. The epididymis is responsible for maturation of the sperms released by the testes so that they acquire motility and become fertile. Therefore knowledge of normal development of testes and epididymis is of utmost importance, as it is essential for clinical evaluation of abnormalities in sexual differentiation in domestic animals. Different environmental and physiological factors may influence normal embryological differentiation of reproductive system which in future is detrimental for reproductive performance. Foetal fluids are necessary for preventing mechanical shock to the developing foetus during entire gestation and for the efficient handling of foetal waste products.

The present study was undertaken to study gross morphology, histogenesis and histochemistry in testis and epididymis of 68 Gaddi sheep foetii collected from slaughter houses in and around Palampur. The samples were divided into four stages based on their age in days obtained after measuring their CRL viz., Stage I (31 to 60 days of fetal age), Stage II (61 to 90 days of fetal age), Stage III (91 to 120 days of fetal age) and Stage IV (121 days till term). The position and shape of testes and epididymis were estimated in all the foetii but the biometrical observations of epididymis could not be observed in foetii of less than 63 days of foetal age due to very small size of epididymis. So these observations were recorded after 65th day of gestation i.e. stage II.

Grossly, the testes were located under mesonephric kidneys and were adhered to its ventral surface in Gaddi sheep foetii of 36 to 41 days of foetal age. The testes were located in the caudo ventral part of the abdominal cavity on 45th day of gestation. The testes were oblong in shape. The metanephros had grown and the mesonephros was undergoing atrophy. The testis continued their migration in the abdominal cavity and entered the inguinal canal along with peritoneal covering on 81st day of gestation. The testes
continued their migration in the canal up to 96 days of gestation and entered the scrotum on 115\textsuperscript{th} day of gestation. The testis remained in the middle of the scrotum till 137\textsuperscript{th} day of gestation and on 140\textsuperscript{th} day of gestation the testis further migrated downwards into the scrotum and settled there.

The size of the testis did not vary significantly between right and left sides among different stages of gestation, however there was continuous and significant increase in the size of the testis with the progression of foetal age.

The length and breadth of the scrotum did not vary significantly between right and left sides. As the gestation progressed, the length of the scrotum increased significantly from stage I to IV of gestation. The breadth of the scrotum increased significantly from stage I to III and then remained consistent at stage IV of gestation.

The epididymis appeared as a straight elongated tubular structure adjacent to the testis as epididymal duct from 45\textsuperscript{th} to 63\textsuperscript{rd} day of embryonic life. In the abdominal cavity, the epididymal duct was located at dorso lateral border of the testicles, which were horizontal in direction. The duct along with testis started migrating from the abdomen towards the inguinal region on 47\textsuperscript{th} day of gestation. The epididymal duct was distinguishable into caput, corpus and cauda regions on 70\textsuperscript{th} day of gestation. The caput occupied the cranial pole of testis as a nodular structure, the corpus appeared as a flat tubular structure and the cauda occupied the caudal pole of testis. On 81\textsuperscript{st} day of gestation when the testes along with epididymis entered the inguinal canal its position was oriented in vertical axis. Thus due to change in axis of testis from horizontal to vertical the epididymis came to lie on caudo lateral border of the testis from this stage onwards. This orientation is maintained till rest of life. The epididymis finally entered the scrotum on 115\textsuperscript{th} day of gestation and then almost settled in the scrotum around 140\textsuperscript{th} day of foetal age. The length, breadth and thickness of epididymis increased with the progression of gestation in Gaddi sheep foeti.

Grossly the gubernaculums appeared as a cylindrical gelatinous mass which extended from caudal pole of the testes and tail of the epididymis to scrotal sac. Gubernaculum tissue continued to swell and on 81\textsuperscript{st} day of gestation it was well demarcated in the inguinal canal whereas was visible in the scrotum on 86\textsuperscript{th} day of gestation. The gubernaculum regressed and appeared as knob like structure which would shrink further to form the future proper ligament of testis, ligament of tail of epididymis and scrotal ligament.
The indifferent gonad developed from the genital ridge in Gaddi sheep foetii. The outer lining of indifferent gonad was found to be simple squamous type at 37th day of gestation. The simple squamous epithelium changed to low cuboidal type of epithelium except towards the mesothelium of the mesonephros on 46th day of gestation. The germinal epithelium became simple cuboidal and had similar characters on 63rd day of gestation.

The tunica albuginea just beneath the germinal epithelium was composed of few layers of mesenchymal cells, fibroblasts and small capillaries on 46th day of gestation. The tunica albuginea was distinguishable in outer thicker tunica fibrosa and inner thin tunica vasculosa on 50th day of gestation which was demarcated clearly on 70th day of gestation. The fibrous layer of the capsule became compact and folded with an increase in the number of blood vessels in tunica vasculosa on 115th day of gestation. The reticular fibers and collagen fibers were abundant. The connective tissue trabeculae which originated mainly from the tunica albuginea layer on 60th day of gestation were seen clearly on 137th day of gestation. They invaginated into the testicular parenchyma dividing it into lobules containing developing sex cords. There was a progressive increase in average thickness of both the layers of the tunica albuginea with increase in the age of Gaddi sheep foetii.

The seminiferous tubules were first observed on 46th day of gestation at the peripheral region of the developing gonad where as the inner region had network of polygonal mesenchymal cells and thin blood vessels. The testicular parenchyma was subdivided into two zones with oval to slightly elongated tubules in the outer zone and elongated tubules in the inner zone on 47th day of gestation. The peritubular cells surrounding the sex cords were first observed on 47th day of gestation. The convolution started in the developing sex cords on 61st day of gestation. The diameter of the sex cords increased progressively from stage I to IV of gestation.

The large cells were first observed on 46th day of gestation in the testis. These cells were spherical or oblong in shape with distinct cell boundaries. The cytoplasm was vacuolated and weakly eosinophilic. There was no significant variation in the diameter of the large cell, its nucleus and nucleolus between right and left testis among different stages of gestation. The diameter of the large cell and its nucleus increased significantly from stage I to II of gestation, then decreased at stage III and again increased significantly at stage IV of gestation in right as well as left testis. Diameter of the nucleolus remained consistent during different stages of development in Gaddi sheep foetii.
The small cells were first observed on 46th day of gestation. These cells were spherical oval or irregular in shape and were located peripherally close to the basement membrane of developing sex cords. The cytoplasm of such cells was relatively more eosinophilic than that of the large cells. There was no significant variation in the diameter of the small cell, its nucleus and nucleolus between left and right testis within each stage of gestation. The diameter of the small cell and its nucleus increased from stage I to IV of gestation in right as well as left testis. Diameter of the nucleolus remained consistent during different stages of development.

The sertoli cells were first observed in Gaddi sheep foetal testis on 46th day of gestation located among small cells of the sex cords. They were roughly pyramidal in shape with indistinct cell boundaries. The length of the sertoli cells increased significantly from stage I to II of gestation, decreased at stage III and thereafter increased significantly again at stage IV of gestation in right as well as left testis. The diameter of the nucleus and nucleolus remained consistent during different stages of gestation in right as well as left testis.

The leydig cells were first observed in Gaddi sheep foetal testis on 46th day of gestation. The interstitium expanded due to differentiation of mesenchymal cells into leydig cells on 60th day of gestation. There was no significant variation in the diameter of the leydig cell, its nucleus and nucleolus among right and left testis within each stage of gestation of developing sheep foetii. The diameter of the leydig cell and its nucleus increased significantly with the advancement of gestation from stage I to IV in right as well as left testis in Gaddi sheep foetii. The diameter of the nucleolus remained consistent during different stages of gestation in right as well as left testis.

The mediastinum testis was observed in the foetal testis of Gaddi sheep on 46th day of gestation as an area devoid of sex cords. In the area of mediastinum testis the rete tubules appeared as clusters of cells on 70th day of gestation. The lower portion of few of the radially directed, solid sex cords near the rete tubules began to form the future tubuli recti at 70th day of gestation.

The ductus epididymis developed from the mesonephric duct which was formed by the growth of caudal end of the pronephric duct. The cranial tip of mesonephric duct appeared as round to oval tubular structure near the caudal end of mesonephric diaphragmatic ligament.

The epididymal duct was surrounded by irregularly arranged tissue at 61st day of gestation and got arranged in a regular manner depicting the formation of future tunica
albuginea at 65\textsuperscript{th} day of gestation which was further differentiated into tunica fibrosa and tunica vasculosa by 70\textsuperscript{th} day of gestation. The connective tissue septae originated from the tunica albuginea and divided the epididymis into lobules at 115\textsuperscript{th} day of gestation.

There was no significant variation in the capsular thickness of the epididymis between right and left testis within each stage of gestation in developing Gaddi sheep foetii. The capsular thickness of the epididymis increased from stage II to IV of gestation in right as well as left testis of Gaddi sheep foetii.

The connective tissue septae originated from the tunica albuginea and divided the epididymis into lobules at 115\textsuperscript{th} day of gestation. There was no significant variation in the capsular thickness of the epididymis between right and left testis within each stage of gestation in developing Gaddi sheep foetii. The capsular thickness of the epididymis increased from stage II to IV of gestation in right as well as left testis of Gaddi sheep foetii.

The intertubular stroma comprised of mesenchymal cells with undifferentiating smooth muscle cells, fibroblasts, blood vessels, collagen fibers and reticular fibers. There was no significant variation in the intertubular distance among right and left epididymis within each stage of gestation in Gaddi sheep foetii. However it increased significantly from stage II to III and thereafter decreased at stage IV of gestation in both right and left epididymis.

The tubular and luminal diameter did not vary significantly between right and left epididymis in each stage of gestation in Gaddi sheep foetii. With the progression of gestation the tubular as well as luminal diameter increased significantly from stage II to III and thereafter decreased at stage IV of gestation in both right and left epididymis.

The lining epithelium of epididymal tubules was tall cuboidal with distinct basement membrane at 70\textsuperscript{th} day of gestation. The lining epithelium contained oval to elongated nuclei located towards the apical surface. The epithelial height of the epididymis showed no significant variation between right and left testis of each stage in developing Gaddi sheep foetii. With the progression of gestation, the epithelial height of the epididymis increased significantly from stage II to III and thereafter no significant change was observed at stage IV of gestation in Gaddi sheep foetii.

The epididymal tubules were surrounded by 7 to 8 layers of mesenchymal cells at 70\textsuperscript{th} day of gestation. The number of layers increased to 12 to 14 at 99\textsuperscript{th} day of gestation which started to differentiate into peritubular smooth muscle cells at 137\textsuperscript{th} day of gestation. The epididymal duct showed 5 to 6 layers of differentiated peritubular smooth muscle cells at 140\textsuperscript{th} day of gestation. There was no significant variation in the thickness of peritubular cellular layer between right and left epididymis in each stage of gestation in developing Gaddi sheep foetii, but it increased significantly from stage II to III and thereafter decreased at stage IV of gestation in both right and left epididymis of Gaddi sheep foetii.
A weak to moderate PAS reaction for glycogen was observed in large cells and rete testis, moderate to strong in germinal epithelium, tunica albuginea, small cells, sertoli cells, peritubular cells and leydig cells and strong reaction was observed in the basement membrane of seminiferous tubules of testis in Gaddi sheep foetii.

A weak alcian blue reaction for acidic mucopolysaccharides was observed in large cells, small cells and sertoli cells, weak to moderate in leydig cells, moderate to strong in germinal epithelium, tunica albuginea and rete testis and strong reaction was observed in the basement membrane and peritubular cells.

A weak to moderate concentration of basic proteins was observed in rete testis, moderate concentration in large cells, small cells, sertoli cells and leydig cells and moderate to strong in germinal epithelium, tunica albuginea, basement membrane and peritubular cells.

The germinal epithelium, tunica albuginea and basement membrane showed negligible reaction for sudanophilic lipids where as testicular parenchyma, mediastinum testis and interstitial space showed weak to moderate reaction in stage I and II of gestation in Gaddi sheep foetal testis. In stage III of gestation a weak reaction was observed in germinal epithelium, tunica albuginea, basement membrane, gonocytes and sertoli cells.

In the epididymis of Gaddi sheep foetii a weak concentration of neutral mucopolysaccharides was observed in the capsule, moderate in peritubular connective tissue layer and strong in the basement membrane, lining epithelium and sub apical surface of the lining epithelium.

A weak concentration of acidic mucopolysaccharides was observed in the lining epithelium and the sub apical surface of the epithelium and moderate in the capsule, peritubular connective tissue layer and the basement membrane of the epididymis.

A weak concentration of basic proteins was observed in the capsule and sub apical surface of the lining epithelium and moderate in the peritubular connective layer and the basement membrane of the epididymis.

A weak reaction for lipids was observed in the capsule, basement membrane, lining epithelium and sub apical surface of the epithelium in the epididymis of Gaddi sheep foetii.

The level of various macro and micro minerals, biochemicals and the activity of various enzymes was analyzed in the foetal fluids (allantoic and amniotic) of Gaddi sheep foetii.
Conclusions

Testis

1. All the gross biometrical parameters (length, breadth and thickness) increased with the increase in age of the fetus and there was no significant variation in the gross biometry of left and right testis.
2. The testes started descending into the inguinal canal on 83\textsuperscript{rd} day of gestation and started entering into the scrotum on 95\textsuperscript{th} day of gestation and finally reached the scrotum on 140\textsuperscript{th} day of gestation.
3. The differentiation of cortex and medulla in the indifferent gonad was observed on 37\textsuperscript{th} day of gestation.
4. The differentiation of tunica albuginea and seminiferous tubules containing large, small and sertoli cells was observed on 46\textsuperscript{th} day of gestation.
5. The differentiation of tunica albuginea into tunica fibrosa and tunica vasculosa was evident on 50\textsuperscript{th} day of gestation.
6. The connective tissue trabeculae divided the testicular parenchyma into lobules which contained developing sex cords on 60\textsuperscript{th} day of gestation.
7. The process of convolution started in the developing sex cords on 61\textsuperscript{st} day of gestation and seen clearly 137\textsuperscript{th} day of gestation.
8. The testicular capsule and connective tissue trabeculae contained abundant reticular and collagen fibers in stage IV of gestation.
9. The diameter of seminiferous tubules and of different cells observed in the tubules increased with the progression of gestation.
10. The neutral and acidic mucopolysaccharides were strongly demonstrable in the germinal epithelium, basement membrane and peritubular cells than tunica albuginea and seminiferous tubules. The basic proteins showed moderate to strong reaction and sudanophilic lipids showed weak reaction in different components of testes.

Epididymis

1. The epididymal duct / mesonephric duct appeared grossly as a straight elongated tubular structure adjacent to the testis on 45\textsuperscript{th} day of embryonic life.
2. The epididymal duct was divided grossly into caput, corpus and cauda regions on 70\textsuperscript{th} day of gestation.
3. The position of the epididymis was abdominal on 45th day of gestation, by day 81 of gestation it reached inguinal region and on 115th day of gestation it entered the scrotum.

4. The gubernaculae appeared as a cylindrical gelatinous mass and was visible in the scrotum on 86th day of gestation and appeared as a regressed knob like structure on 99th day of gestation.

5. The gross parameters (length, breadth and thickness) increased with increase in age of Gaddi sheep foetii.

6. The formation of the tunica albuginea was observed on 65th day of gestation and its differentiation into tunica fibrosa and tunica vasculosa was evident on 70th day of gestation.

7. The connective tissue septae originated from the tunica albuginea and divided the epididymis into lobules at 115th day of gestation.

8. The capsule contained abundant reticular and collagen fibers on 137th day of gestation.

9. The tubular and luminal diameter increased significantly from stage II to III and thereafter decreased at stage IV of gestation.

10. The lining epithelium of epididymal tubules was tall cuboidal type and the height of the epithelium increased with the progression of gestation.

11. A strong PAS reaction was observed in the basement membrane, lining epithelium and sub apical surface of the lining epithelium and moderate reaction in the capsule of the epididymis in Gaddi sheep foetii.

12. The acidic mucopolysaccharides were present in moderate concentration in capsule, basement membrane and peritubular connective tissue layer. The basic proteins showed moderate to strong reaction and sudanophilic lipids showed weak reaction in different components of epididymis.

**Mineral and Biochemical Analysis**

**Allantoic Fluid**

1. In the allantoic fluid of Gaddi sheep the concentration of glucose, total proteins, creatinine and triglycerides decreased in stage IV as compared to stage I of gestation where as the concentration of albumin, urea, uric acid, cholesterol, total and direct bilirubin increased.
2. The concentration of potassium, calcium, zinc and magnesium increased and the level of sodium and chloride decreased however the concentration of phosphorus, copper and iron remained consistent as the gestation progressed.

3. The concentration of AST and ALT increased while that of ALP decreased with the advancement of gestation.

**Amniotic Fluid**

1. In the amniotic fluid of Gaddi sheep the concentration of uric acid, urea and cholesterol increased; glucose, creatinine, direct bilirubin and triglycerides concentration decreased and total proteins, albumin and total bilirubin remained consistent with progression of gestation.

2. The concentration of phosphorus, iron and magnesium increased and the level of sodium, potassium, zinc and chloride decreased however the concentration of copper remained consistent as the gestation progressed.

3. The concentration of AST and ALT decreased while that of ALP increased with the advancement of gestation.