CHAPTER 2

REVIEW OF LITERATURE

2.1. Gross Morphological studies

2.1.1. Gross Morphology of Testes

Attal (1969) observed that the testes were slightly larger and more rounded than ovaries in sheep at 35 days of gestation and there was an abrupt increase in the testicular weight after 70 days of embryonic life.

Dehkordi et al. (2008) observed that the length of the right testis (0.18 cm – 0.50 cm) was non significantly greater than that of the left testis (0.17 cm – 0.53 cm) in sheep foeti at 41 to 86 days of gestation. The width of the left testis (0.18 cm – 0.32 cm) was numerically greater than that of the right (0.16 cm – 0.31 cm) at 41 to 70 days of gestation, however at 71 to 86 days of embryonic life the width was more in the right testis (0.37 cm) as compared to that of the left one (0.34 cm) in sheep. They further stated that difference of the weight between right and left testis was not statistically significant, however, the difference with regard to thickness between right (0.20 cm – 0.34 cm) and left testis (0.14 cm – 0.42 cm) was statistically significant in sheep foeti at 41 to 86 days of gestation.

Amin and Alwan (2010) demonstrated that the weight of the foetal testes and length of the foetal scrotum were steadily increased in sheep from 47 days to 153 days of gestation.

Dhande et al. (2006) stated that the testes were oval and the average weight, length, width and circumference were 4.3 gm, 13.65 mm, 6.6 mm and 24.10 mm, respectively at the completion of the term, in sheep foetus.

Kaur (2006) reported that the shape of the both testes varied from rounded at 42 to 110 days to oval at 122 to 163 days of gestation and thereafter they were elongated in shape throughout gestation period. The right testis was found to be longer (1.60 ± 0.12 cm), broader (1.03 ± 0.10 cm) and thicker (1.05 ± 0.18 cm) than the left testis (1.53 ± 0.12 X 0.93 ± 0.12 X 0.93 ± 0.17 cm) at 173 days of gestation and onwards in buffalo foeti. The average weight of the right testis was found to be 1.54 ± 0.25 gm at 122 to 163 days of foetal age and 2.88 ± 0.43 gm at 173 days and onwards of gestation and in the left testis it was 1.39 ± 0.33 gm and 2.72 ± 0.41 gm respectively. Thus, she concluded that the right testis was heavier than the left testis throughout embryonic life. Similarly Mathur et al. (2005) observed no significant difference between right and left testes with respect to length, width, thickness and weight during full gestation period in Frieswall bulls.
Abdel-Raouf et al. (1974) stated that the weight of the testis increased to about 63 fold in buffalo foetii till 180 days of gestation and was more correlated to the body weight than the crown - rump length.

Singh (1996) observed the testicular length and width to be 2.00 ± 0.147 cm and 1.00 ± 0.06 cm, respectively in newly born buffalo calves and concluded that the development of the testes was slower and extended over longer period in the buffalo.

2.1.2. Gross morphology of epididymis

The bovine epididymis was comprised of several vasa efferentia and a long, coiled ductus epididymis. During embryonic life the vasa efferentia is derived directly from the mesonephric tubules and ductus epididymis is differentiated from the mesonephric duct (Noden and de Lahunta 1985 and Gunther 1995). Nielsen and Torda (1983) observed in rabbit foetuses that epididymis located superiorly on the testis and at 24 days of gestation there was beginning of formation of cap over the testis, occupying a variable amount of the superior pole. From 26th day of gestation to 30th day of gestation the cap was well formed over the superior pole of the testis with increase in size of epididymis.

Cunha et al. (2005) observed that the coiling in Wolfian duct in male foetus of spotted hyena started at 80th day of gestation.

2.2. Histogenesis

2.2.1. Testes

(i) Formation of the genital ridge

The gonads arose as a ridge like thickening called the gonadal ridge or germinal ridge or genital ridge on the ventromedial face of the mesonephros in embryos (Patten 1953).

Gonadal ridge develops as a thickening of coelomic epithelium and condensation of underlying loose mesenchyme along the ventro cranial area of the mesonephros as reported by Witschi (1951), Hamilton and Mossman (1972) and Merchant (1975). However, George and Wilson (1994) stated that in mammals gonadal ridge originated as a paired mesenchymal primordia projecting on each side of the dorsal mesentery, from the medial aspect of mesonephros.

Harshan (1986) stated that gonadal primordial was formed as a thickening of mesothelium along the ventral aspect of mesonephros in goat embryos at 17 days of gestation.

Singh et al. (1979) and Farooqui et al. (2011) observed that the gonadal ridge extended from 5 – 12 body segments in goat embryos at 23 days of gestation and had focal
accumulation of mesenchymal cells covered by a simple squamous epithelium which continued at both the ends as mesothelium of the mesonephros. The bovine gonadal ridge was found to be covered by two layered columnar epithelium with elongated or round nuclei which appeared at 27 – 31 days post conception (Schrag 1983 and Wrobel and Sub 1998).

Russe (1991) noticed the gonadal ridge in sheep embryos at 28 days post conception.

Erickson (1966) and Ghannam and Deeb (1967) observed the genital ridge on 32\textsuperscript{nd} day of gestation in bovine embryo and on 31\textsuperscript{st} day of gestation in Egyptian buffalo embryos.

(ii) Indifferent Gonad

The foetal gonad first appeared as a tiny swelling that grows along the coelomic surface of the mesonephros. It was histologically identical in both sexes and was named as the indifferent or bipotential gonad (Capel 2000). The indifferent mammalian gonad was defined as a modified homologue of the pronephros by Wrobel and Sub (2000) and was situated in the zone of pro/mesonephric overlapping.

Zamboni and Upadhyay (1982) observed that the gonadal ridge assumed the characteristics of a sexually indifferent gonad in sheep foetus on of 28\textsuperscript{th} day of development, however, Hejazi et al. (2011) observed the gonad adjacent to the ventromedial part of mesonephros for the first time in sheep foeti at 37.82 days of gestation.

Gier and Marion (1970) reported that the growth pattern of gonadal ridge resulted into the formation of globular gonad by 32 days in dog and 39 days in ox. Wrobel and Sub (1998) observed unevenly distributed primordial germ cells in the sexually indifferent gonad in bovine embryo at 32 - 39 days of prenatal life.

(iii) Sexual Differentiation

Schrag (1983) noticed that the first visible event leading to testicular differentiation began with the development of tunica albuginea and formation of testicular or primitive sex cords in mammals. During the process of sexual differentiation the epithelium of the genital ridge which formed the surface of the gonad is known as the cortex of gonad and the primitive sex cords constituted its medulla (Balinsky 1970 and Lathshaw 1987).

Schlegel et al. (1967) observed that gonads undergo sexual differentiation into testis through development of the primitive medulla and regression of the primitive cortex.
Jost (1972a) and Peters (1976) observed that the gonads differentiated earlier in males than in females.

Attal (1969) found that the differentiation of primordial gonad in the ovine foetus took place 35 days after copulation. Singh et al. (1979) stated that the differentiation of testis started in goat embryo at 39.33 days of gestation and was evidenced by the formation of tunica albuginea and seminiferous cords which became tortuous and joined the rete tubules at 98.65 days of gestation. Russe (1991) observed the process of testicular differentiation at 31 days post conception in sheep embryo.

Bascom (1923) distinguished sex on the basis of appearance of the germinal epithelium and tunica albuginea at 39.9 days of gestation in bovine embryo. Ghannam and Deeb (1967) reported that the sex of foetus was evident in Egyptian buffalo embryo at 37.65 days of gestation while Gier and Marion (1969) and Sharma and Lukuteke (1979) reported sexual differentiation changes in the gonads of bovine at 42 days and at 50 days post conception.

The sexual differentiation in equine foetii was completed by day 30 of intrauterine life as reported by Sakai (1955) and Russe (1991). Gier and Marion (1969) observed the differentiation changes in the gonad at 33 days post conception in pigs.

Pelliniemi (1975a) reported that in pig foetus at 26 days of gestation the testes were distinctly differentiated into four tissues i.e. surface epithelium, testicular cords, interstium and mesenchyme.

(iv) Tunica albuginea

Singh et al. (1979) stated that the formation of tunica albuginea began when mesenchymal cells beneath the mesothelium of developing gonad got condensed into fibroblasts in the goat embryos of 47 days of gestation. These fibroblasts organized into definite layers of tunica albuginea with trabecular extension deep into the testis in goat foeti of 48 days of gestation.

Pachpande et al. (2006) observed that the tunica albuginea became thicker as the gestation advanced and showed progressive differentiation of the fibroblast and fibromuscular character during foetal life in sheep.

Dekhordi et al. (2008) described tunica albuginea to be a homologous layer consisting mainly of undifferentiated mesenchymal cells and was covered externally by a layer of coelomic epithelium. They found that the diameter of the capsule was 7.21 µm at 41 – 45 days, 30.79 µm at 46 – 63 days, 48.62 µm at 64 – 70 days and 50.61 µm at 71 – 86 days of gestation in sheep foeti.
Farooqui et al. (2012) observed the capsule just beneath the germinal epithelium and found that it was composed of 2 – 4 layers of mesenchymal cells, fibroblasts and small capillaries in goat at 44\textsuperscript{th} day of gestation. The further stated that the average thickness of tunica fibrosa and tunica vasculosa increased from 17.94 ± 2.00 µm to 101.70 ± 3.65 µm and 12.30 ± 1.73 µm to 76.16 ± 3.44 µm, respectively at 31 – 60 days and full term of gestation.

Matschke and Ericksen (1969) reported the appearance of tunica albuginea in bovine foetal testis at 36 days of embryonic life.

The capsule differentiated into outer fibrous layer (tunica fibrosa) and inner vascular layer (tunica vasculosa) in buffalo foetii at 65 days of gestation as reported by Kaur (2006). She observed elastic fibres in the tunica intima of blood vessels in tunica vasculosa and in testicular artery and nerve fibres in the testicular nerve at 272 days of gestation. She found an increase in thickness of tunica vasculosa from 23.68 ± 1.67 µm to 109.55 ± 2.85µm in right testis and from 25.15 ± 1.68µm to 112.77 ± 2.26 µm in left testis and the thickness of tunica fibrosa varied from 41.75 ± 2.29 µm to 167.78 ± 2.37 µm in right testis and from 42.41 ± 2.27 µm to 168.80 ± 2.19µm in left testis.

Gier and Marion (1970) observed the appearance of tunica albuginea at 30, 33 and 41th day of gestation in horse, dog and ox, respectively.

(v) Septula testis

Farooqui et al. (2012) stated that the mesenchymal cells from the capsule along with large cells and small blood vessels invaginated in between the developing sex cords and formed the septula testis in goats at 44\textsuperscript{th} day of gestation. These septae contained reticular fibres from 48\textsuperscript{th} day of gestation and collagen fibres appeared at 121 day onwards of gestation.

(vi) Sex cords

Burns (1961) reported that the primary sex cords in amniotes proliferated from the germinal epithelium and formed the medullary cords. The medullary cells developed further and became testicular cords. Several investigators used the term sex cords for seminiferous tubules until these were luminated (Hooker 1944; Santamarina & Reece 1957; Abdel Rauf 1960; Goyal & Dhingra 1973 and Chandra Pal 1976).

Singh et al. (1979) observed the process of formation of sex cords as cellular aggregation beneath the developing tunica albuginea in goat foetal testis at 47\textsuperscript{th} day of gestation. They were found to be composed of small rounded cells with barely discernible
outlines and large clear cells with distinct boundaries. They also found increase in seminiferous tubular diameter with the progression in age of foetuses.

Baishya et al. (1987) stated that the diameter of seminiferous tubules did not reveal much variation between right and left testes (39.67 ± 2.73 µm and 39.37 ± 2.68 µm) in Assam goat however they observed an increase in the tubular diameter from 15 to 60 days (36.87 ± 13.10 µm to 50.14 ± 13.89 µm) with a subsequent decrease at 61 days (34.47 ± 11.95 µm) and onwards.

Farooqui et al. (2012) reported that the mean diameter of sex cords measured 28.66 ± 1.96 µm, 41.55 ± 1.42 µm, 40.06 ± 0.88 µm and 42.21± 1.66 µm in goat foeti at 31 – 60 days, 61 – 90 days, 91 – 120 days and 120 – till term, respectively. They further stated that sex cords contained two types of cells – large cells and small mesenchymal cells arranged in a chain like manner at 44th day of gestation in goats. Baishya and Vyas (1990) observed the presence of large cells and small cells in sex cords since 103 days of foetal life in Surti buffalo.

Presence of large and small cells in developing seminiferous tubules was earlier reported in embryos of cattle (Santamarina & Reece 1957), pig (Moon & Hardy 1973), goat (Singh et al. 1979) and human beings (Arey 1966 and Copenhaver et al. 1978).

Santamarina and Reece (1957) recorded much variation in tubular growth in different breeds of bovine testes at prenatal and postnatal stages and concluded that tubular growth of individual animal depended much upon the stage of development and not upon breed or age.

Kaur (2006) observed seminiferous tubules first at the gonadal periphery where the inner part consisted of network of polygonal mesenchymal cells and thin walled blood vessels in buffalo foetii of 65 to 74 days of gestation. The average diameter of longitudinal seminiferous tubules in right and left testis increased from 114 days (102.36 ± 3.95µm and 103.89 ± 3.57µm) to 164 days (126.60 ± 1.63µm and 121.89 ± 1.77µm) and onwards in buffalo foetii. The average diameter of rounded tubules increased from 38.73 ± 1.47µm to 54.33 ± 1.42µm and 39.39 ± 1.44 to 55.34 ± 1.35µm µm in right and left testis respectively in buffalo foeti from 114 days to 164 days of gestation.

(vii) Gonocytes

Lacy (1962), Hugon and Bogers (1966) and Reddy and Svoboda (1967) reported that in the seminiferous tubules fetal germ cells and sertoli cells were seen and resorption of degenerating fetal germ cells by sertoli cells resembled the resorption of degenerating spermatogenic cells in the adult testis.
Wartenberg et al. (1971) reported that the gonocytes could be differentiated from supporting cells owing to their larger size, round shape and relatively pale cytoplasm. The gonocytes enclosed within testicular cords were responsible for synthesis of testosterone (Jost 1972b).

Gondos (1977) described that the germ cells of undifferentiated foetal gonads had primitive germ cells i.e. gonocytes when the germ cells were located in centre of sex cords and prespermatogonia when germ cells migrated to the periphery of the tubule and was in contact with basement membrane of tubules.

Primordial germ cells were reported as large cells in pig (Patten 1948), goat (Singh et al. 1979 and Farooqui et al. 2012) and human beings (Copenhaver et al 1978). Farooqui et al. (2012) had reported small cells as mesenchymal cells in goat.

Singh et al. (1979) reported that the sex cords were composed of small rounded cells with barely discernible outlines and large clear cells with distinct boundaries in goat foetal testes at the age of 48 days onwards. The former were found to be arranged at the periphery, while the later occupied the centre of cords.

Baishya et al. (1987) found that the tubular lumen contained gonocytes and prespermatogonia upto 60 days of gestation in Assam goat. At 61 days and onwards these cells were found to be absent and the seminiferous epithelium showed cells of further developmental stages viz: spermatogonia and primary spermatocytes.

Farooqui et al. (2012) stated that the large cells were spherical or oblong in shape with distinct cell boundaries and their spherical or ovoid vesicular nuclei were eccentrically placed in goat foetal testis. They found that the average diameter of large cells and their nucleus increased from 8.5 ± 0.14 μm to 14.82 ± 0.29 μm and 5.33 ± 0.28 μm to 7.88 ± 0.61 μm respectively in goat foeti from 31 days to 120 days of gestation. They further stated that the small cells were spherical, oval or irregular in shape and were located at the periphery close to the basement membrane. The average diameter of such cells and their nuclei increased from 5.64 ± 0.45 μm to 12.68 ± 0.29 μm and 4.3 ± 0.16 μm to 7.77 ± 0.17 μm respectively in goat foeti from 31 days to 120 days of gestation.

Wrobel (2000) reported that the germ cells with high proliferation rate were observed from day 50 post coitum to day 80 post coitum in bovine fetal testis and these cells were in transition from primordial germ cells to prespermatogonia. From day 80 postcoitum to 15 postnatal weeks the germ cells present were identified as prespermatogonia.
Kaur (2006) reported that gonocytes were demonstrable in the centre of seminiferous tubules of buffalo foetii at 74 days of gestation. She further stated that the size of gonocytes and their nucleus did not vary significantly with respect to age and testis of the buffalo foetii.

Baishya and Vyas (1990) observed the presence of germ cells in Surti buffalo at 103 days of foetal age. They found constant increase in the average cells population of small cells (5.00 to 13.00) and large cells (1.00 to 4.00) per seminiferous cords from 69 days to 103 days and onwards of gestation.

Abdel Raouf et al. (1974) found that the number of gonocytes increased with advancing age in buffalo foetal testis but at later stages of gestation some of the gonocytes showed degenerating changes. Abd – Elmaksoud (2005) reported the maximum number of gonocytes (1.16 ± 0.38 µm) in the later stages of foetal development in bovines.

Santamarina and Reece (1957) and Goyal and Dhingra (1973) found origin of spermatogonia from the tubular small cells and not from the large cells which were described as prirmodial germ cells or gonocytes. However, with the advent of different advanced studies, gonocytes had been reported to give rise to adult germ cells (Courot et al. 1970). It was also revealed that transformation of gonocytes into spermatogonia occurred through an intermediate prespermatogonial stage (Gondos 1977 and Guraya 1980).

(viii) Supporting or Sustentacular or Presumptive or Presertoli cells

Magre and Jost (1980) reported that the appearance of presumptive sertoli cells is one of the first identifiable events in testicular differentiation in mammals. Magre et al. (1980) observed that the differentiation of the sertoli cells involved changes in cytoplasm and organelles, typical junctions and microfilaments toward the external surface of the seminiferous cord.

Martineau et al. (1997), Karl and Capel (1998) and Tilmann and Capel (1999) observed that cells derived from the mesonephros or coelomic epithelium or both can contribute to the sertoli cells populations.

Upadhyay et al. (1981) reported a uniform origin of the precursors of sertoli cells from the mesonephros of the foetal sheep. Singh et al. (1979) reported that the sertoli cells were present as small rounded cells with barely discernible outlines arranged at periphery of the sex cords in goat foetal testis of 48 days.

Farooqui et al. (2012) found that the pre sertoli cells were located among the small cells of the sex cords having a roughly pyramidal shape with indistinct cells boundaries in
goat foeti at 56th day of gestation. They further reported that the height of the sertoli cells and its nucleus varied from 6.0 – 8.5 µm and 3.9 – 7.4 µm at foetal age of 30 – 60 days; 10.5 – 19.5 µm and 4 – 13.5 µm at 60 – 90 days of age; 10.5 – 15 µm and 4.5 – 7.5 µm at 90 – 120 days of age and 10.5 – 18 µm and 6 – 9 µm at foetal age of 120 days and onwards.

Hochereau de Reviers et al. (1995) observed a positive linear relationship between the logarithmic values of age and total number of sertoli cells and germ cells per testis in sheep foetus.

Matschke and Erickson (1969) observed the supporting cells adjacent to the basement membrane in bovine foetal testis at day 70 of the gestation period. Schrag (1983) observed that in bovine, the pre sertoli cells were columnar in shape. Towards the end they developed into densely packed concentric or spiral accumulated piles, whose content increase continuously. The pre sertoli cells were first observed at the periphery of seminiferous tubules in buffalo foetii of 65 days by Kaur (2006). The maximum number of pre sertoli cells was found to be 16 to 20 per cross-sectional area and their number considerably increased with the progression of fetal life (Abd – Elmaksoud 2005 and Kaur et al. 2008).

Bardin et al. (1994) observed that the differentiation of sertoli cells in vivo and found that their subsequent proliferation during fetal and neonatal life was complex event involving unknown signal for the initiation of differentiation from within the testis. It appeared that once initiated, the process continued in an orderly fashion and completed prior to puberty.

De Kretser and Kerr (1994) reported that the function of fetal sertoli cell was phagocytosis of the degenerating germ cells and secretion of anti mullerian hormone (AMH) responsible for regression of the mullerian duct in male fetus. The basement membrane acted as a uniting media between the pre-sertoli cells and peritubular cells which were responsible for the formation of blood – testicular barrier (Byskov & Hoyer 1994).

(ix) Extracellular matrix: peritubular cells and basal lamina

Tung et al. (1984) reported that basal lamina had possibly developed as a bilateral network of the sertoli cells and peritubular cells. The basal lamina underlie the epithelium of seminiferous tubules was a shared product of sertoli cells (Skinner et al. 1985).
Sinowatz et al. (1987) and Orth (1993) observed that pre sertoli cells appeared to cooperate with a sub population of mesenchymal cells, presumably the future peritubular cells, to produce the extra cellular material surrounding both cell types in young foetus.

Martineau et al. (1997) reported that peritubular myoid cells arose from a population of cells that migrated into the genital ridge from the mesonephros.

Bustos – Obregon and Courot (1974) reported in sheep that the lamina propria showed clear signs of morphological specialization 21 days after the gonadal differentiation.

In bovine testis, all testicular cords were surrounded by continuous heparin sulphate and laminin positive basal lamina shortly after the testicular differentiation (Wrobel 2000).

(x) **Interstitial tissue**

Witschi (1951) stated that the precursor of leydig cells had a mesonephric origin. The origin of interstitial endocrine cells from the interstitial mesenchymal cells was reported in cattle (Santamarine & Reece 1957 and Gier & Marion 1970), dog (Gier & Marion 1970) and pig (Black & Christensen 1969 and Moon & Hardy 1973).

Holstein et al. (1971) observed that the second component which differentiated from the testicular blastema proper is the interstitial tissue consisting of undifferentiated fibroblast.

Byskov (1986) suggested that most of foetal leydig cells originated by differentiation from mesenchyme like precursors and once differentiated, no longer divide under normal conditions. Leydig cell number was found to be more in foetal testis as compared to testis of neonate and after the neonate period the leydig cell number further increased (Lording and de Kretser 1972; Christensen 1975; Tapanainen et al. 1984; Zirkin and Ewing 1987 and Singh 1996). Wrobel (1990) concluded that mammalian leydig cells emerge in two successive waves; the first leading to foetal generation, second beginning at puberty and leading to adult leydig cell population.

Farooqui et al. (2012) reported that the mesenchymal cells started differentiating into interstitial endocrine cells (leydig cells) located in the interstitial spaces at 44th day of gestation in goats. These cells were found to be present in groups of 2 – 3 cells along with differentiating fibroblasts at 56th day of foetal life. The diameter of leydig cells and its nucleus in goat foetal testis at 31 – 60 days, 61 – 90 days, 91 – 120 days and 121 – till term was 7.58 ± 0.62 µm and 5.41 ± 0.32 µm, 11.78 ± 0.68 µm and 6.0 ± 0.28 µm, 12.58 ± 0.36 µm and 6.66 ± 0.22 µm, 12.66 ± 0.27 µm and 5.83 ± 0.24 µm respectively.
Singh et al. (1979) observed that the indifferent mesenchymal cells occupied the spaces between the seminiferous cords as interstitial tissue in goats at 48 days of gestation. These mesenchymal cells of the interstitial area differentiated as leydig cells at 60 days of gestation. They further stated that the number and size of leydig cells in goat embryos increased with increase in gestation length however they observed a decrease in number of cells in the embryos of more than 115 days. Pachpande et al. (2006) also reported a progressive increase in number and size of the leydig cells as the crown – rump length of the sheep foetus increased.

Wrobel et al. (1988) reported that the intertubular mesenchymal cells were not only the precursors of the leydig cells but also of the peritubular cells and fibrocytes in bovine. The appearance of leydig cells followed that of the testicular cords by about 1 week in bovine (Sinowatz et al. 1987; Russe 1991 and Orth 1993).

Vigier et al. (1976) observed the appearance of interstitial cells 2 – 3 days after the formation of first seminiferous cords in bovine foetus. Schrag (1983), Sinowatz et al. (1987) and Russe (1991) reported that the foetal leydig cells of bovine were firstly recognized in the foetus at 46 days post conception.

Abdel – Raouf et al. (1974) stated that pre leydig and young cells were found in intertubular spaces at 90 days and they differentiated to mature leydig cells at 120 days of gestation in buffalo foetus.

Hullinger and Wensing (1985) related the development of interstitial endocrine (Leydig cells) of the foetal testis in calf with the swelling reaction of the gubernaculums and normal prenatal descent of the testis.

Baishya and Vyas (1990) observed a uniform pattern of distribution of leydig cells with relatively large round to ovoid light nuclei in Surti buffalo up to about 123 days of gestation and after 123 days the leydig cells demonstrated characteristic changes of foamy cytoplasm and condensed darker type of nuclei. They further stated that leydig cells showed a progressive relative increase both in number and size with progress in gestation period and concluded that steroidogenesis might have been started in Surti buffalo foeti from about 123 days and onwards and might have been increased with the advancement of foetal age.

However, Gier and Marion (1970) stated that leydig cells after their first appearance in bovine foetal testis increased progressively both in size and number till birth. They demonstrated plump type distribution of leydig cell in bovine foetus. Guraya (1980) found an increase of testosterone production with corresponding increase in the
number of leydig cells. Similar opinion was also expressed by Hooker (1970), who further contradicted the same stating that the type of leydig cells rather than total number was a significant consideration.

Kaur et al. (2011) reported the first presence of leydig cells in buffalo foetii at 65 days of gestation. Moon and Hardy (1973) reported 3 types of interstitial cells in pig foetus viz. Type A or mesenchymal cells, Type B or immature leydig cells and Type C or mature leydig cells.

(xi) **Mediastinum testis**

Singh et al. (1979) reported that the analagen of mediastinum testes appeared as an area devoid of seminiferous cords, in goat embryo at 48 days of gestation however, Farooqui et al. (2012) observed the same in goats at 44th day of gestation.

(xii) **Rete tubules**

Patten (1953) stated that the rete testis persisted as a network of channels in foetal testis which connected the seminiferous tubules to the efferent ductules in the foetal testis of ruminants, pig and dog. The central most cords of blastema cells (rete blastema) and those nearest to the area of attachment of the testis with the mesonephros, organized into an interconnecting network of cords that did not contain germ cells were termed as the rete testis (Dellmann 1993).

Zamboni and Upadhyay (1982) found that the central region which contained somatic cells became organized into tubuli recti in sheep foetal testis by day 52 of development, however, Sweeney et al. (1997) observed the rete testis in the centre of testis of ovine foetus by day 70 of gestation. Farooqui et al. (2012) observed that at 70th day of gestation in goats, the rete tubules appeared as clusters of 5–6 cells in the area of mediastinum testis. Vacuolation were observed in the central area of developing rete tubules marking the beginning of the lumen formation.

Singh et al. (1979) observed typical rete tubules lined with simple cuboidal epithelium in goat foeti at 106 days of gestation extending towards the deeply presented seminiferous cords. Cuboidal epithelium was also demonstrated in bovine foetal rete testis by Banks (1993) and Abd – Elmaksoud (2005).

Gier and Marion (1970) also described that the analagen of rete testis along the antero–lateral border of the gonadal ridge became evident on the very first day of its development. The small area of the indifferent blastema in the centre of the testis at the age of 26 days represented the region of the future rete testis in pig embryo (Pelliniemi 1975a).
In bovine foetus at 50 – 60 days the extra testicular rete had a blastema like appearance and consisted of irregular cells with abundant glycogen, later on extensions of the extra testicular rete came in contact with efferent ductules and the invading rete cells intermingled with epithelium of efferent ductules and established the urogenital junction (Wrobel and Schimmel 2001).

(xiii) Descent of the testis

Persaud (2003) stated that the testicular enlargement and its movement during dorsal wall of abdominal cavity were affected by the atrophy of the mesonephros. Backhouse (1982) in studies on gubernaculum noted that at the time of migration and testicular descent in the abdominal cavity, mesenchyme of gubernaculum was loosened and simultaneously the scrotum spread. Sadler (2004) reported that gubernaculum was main responsible for testis extraction into scrotum. Baishya & Vyas (1991) stated that each testis descended more towards the inguinal ring at the age of 61 – 90 days of gestation in Surti buffalo foeti. They further described that the descent of testis in caudal direction further towards the inguinal canal was due to the growth of the body & enlargement of adjacent organs. Farooqui et al. (2011) reported that the testes were situated in the inguinal canal from 89th to 95th day of gestation in goat but did not reach the distal end of scrotum up to parturition. Gier and Marion (1970) reported that the testes passed the inguinal ring in the foetal sheep at about 80 days, in bull at 100 – 105 days, in pig at 100th – 110th day and in horse at about 300 days. Ram Kumar et al. (1988) mentioned that the scrotal migration started at 90 days in goat foetus. Arthur (1964) observed descent of the testis into the scrotum in sheep between 2.5 – 3 months. Klonish and Flower (2004) reported that testis descent in pigs, horses, cattle and sheep at birth. Evans and Sak (1973) stated that in calves testis descent to the scrotum at 140 days of pregnancy. Abdel – Raouf (1974) in a study on the evolution of testis in buffalo revealed that testicles at 180 days of pregnancy entered into the scrotum. Bissa et al. (1988) also reported that the testes had not descended in the scrotum in Bikaneri camel calves even at birth.

2.2.2. Histogenesis of epididymis

(i) Ductuli efferentes

a. Embryogenesis

The vasa efferentia were derived directly from mesonephric tubules to which the rete testis was attached (Gier and Marion1969). Marshall et al. (1979) also observed that the ductuli efferentes and caput epididymis derived from the mesonephric tubules in the
rat, whereas Wrobel (2001) and Mohamed (2005) stated that the efferent ductules originated as a new set of secondary mesonephric tubules that grew out from the dorsal aspect of the mesonephric giant corpuscle and were not formed by transformation of primary mesonephric tubules. They also noticed that the efferent ductules joined the extra testicular rete testis at the urogenital junction.

Moustafa and Hafez (1971) reported that the mesonephric tubules were converted into efferent ductules between 70 and 150 days of gestation in bovine foetuses.

Russe and Sinowatz (1991) observed that the ductuli efferentes originated as well developed coiled structure and formed the coni vasculosa in caput epididymis by the fourth month of gestation in bovine foetus.

The establishment of urogenital junction was observed in bovine foetus at about 85 days post conception (Wrobel and Schimmel 2001 and Mohamed 2005). However, Wrobel (2001) found that the urogenital junction was completely formed in bovine foetuses at 150 days post conception by forming the luminized channels of extratesticular rete testis to form end to end anastmosis with efferent ductules.

Goyal (1983) reported the closely packed aberrant ductules in between the lobules of ductuli efferentes in bull. Very short blind ended ductules were also observed in bulls by Amselgruber and Sinowatz (1991).

b. Differentiation of epithelium

Moustafa and Hafez (1971) noticed that efferent ductules were lined by an irregular cuboidal epithelium supported by a prominent basement membrane in bovine foetii between 70 and 150 days of gestation. Russe and Sinowatz (1991) observed that the epithelium of ductuli efferentes contained ciliated and non ciliated cells at the 120 days of gestation in bovine foetus.

Goyal and Hruduka (1981) described three types of non ciliated cells in the lining epithelium of bull and goat as: type I, II and III. Mohamed (2005) reported that efferent ductules were lined by a simple low columnar epithelium with a distinct basement membrane in bovine foeti at 74 to 87 days of gestation which became simple columnar epithelium with ciliated and non ciliated cells at 276 days of gestation.

Bansal and Uppal (2009) found that the number of rounded and oval efferent ductules varied from 16 – 20 in buffalo foetuses at 74 to 112 days of gestation and were lined by simple columnar epithelium with large elliptical nucleus having prominent nucleoli.
c. Peritubular tissue development

The proliferating mesenchymal cells surrounding the epithelium of efferent ductules arranged in several concentric layers at about day 85 of post conception and were considered to be the precursors of the periductular musculature in bovine foetuses (Wrobel 2001).

Mohamed (2005) reported that the periductular mesenchymal cells were concentrically arranged in about 4 cell layers around the efferent ductules with their cytoplasmic processes joining each other in bovine foetuses at 74 to 87 days of gestation. These periductular smooth muscle cells were formed from differentiating mesenchymal cells at 109 to 118 days of gestation. The periductular cells were arranged in 2 to 3 layers of smooth muscle cells after 141 days and onwards of gestation. This showed the decreased pattern of smooth muscle layer formation surrounding the efferent ductules with the increase in the foetal age.

d. Capsule and stroma

Wrobel (2001) reported the presence of nerves in the area of efferent ductules at about 80 days post conception in bovine embryo which was in direct contact with the ductular walls at 130 days post conception. The density of nerve fibres increased with the age of foetus and a continuous dense network of fibres extending into the thick muscular layer around the efferent ductules was noticed at 250 days post conception.

Mohamed (2005) observed that the efferent ductules were organized in the lobules separated from each other by connective tissue septa in bovine foetus from 141 days and onwards of gestation.

Bansal and Uppal (2009) found that the interstitial tissue of vasa efferentia comprised of only mesenchymal cells and blood vessels in buffalo foetus of 74 days of development.

e. Micrometry

Hemeida et al. (1978) noticed that the height of epithelium of ductuli efferentes varied from 20 – 30 µm and the length of cilia was 8 to 13 µm in bull whereas in buffalo bull the height of epithelium of ductuli efferentes was 16 to 24 µm and the height of cilia was 4 to 6 µm (Pal and Bhardwaj, 1989).

Khurana (2000) recorded the tubular and luminal diameter of ductuli efferentes as 52.1 ± 3.4 µm and 28.8 ± 1.7 µm respectively in 6 month old buffalo calf and the cross sectional area of the tubules, lumen and epithelium was 2191 ± 287 µm², 693 ± 82 µm²
and $1498 \pm 257 \, \mu m^2$ respectively. He also observed that the epithelial height was less in the buffalo calf as compared to adult buffalo bull. The height of cilia was $5.2 \pm 1.3 \, \mu m$ in buffalo calf.

(ii) Ductus Epididymidis

a. Embryogenesis

Flickinger (1969) described that mammalian epididymis was formed by the mesonephric duct and some of the mesonephric tubules at the level of developing testis.

Gier and Marion (1969) stated that the epididymal duct and vas deferens were derived from the mesonephric duct, and the attachment of gubernaculum across the mesonephric duct provided the specific demarcation between epididymis and vas deferens. The head of epididymis was formed by vasa efferentia and associated epididymal duct in the most mammals except in rat where it was formed entirely by epididymal duct.

In the bovine foetus, epididymal duct developed from that portion of the mesonephric duct where the caudal efferent ductules opened and the epididymis started to develop from the mesonephric duct at 60$^{\text{th}}$ days post conception (Russe and Sinowatz 1991). The epididymal duct lengthened to convolute strongly at the fourth month of gestation and this convoluted duct formed the caput, corpus and cauda epididymis.

Sharma (2010) observed that the epididymal duct appeared as a straight elongated tubular structure in buffalo foeti of 69 to 85 days of gestation and migrated completely into the scrotum along with testis at 162 days of gestation. They further stated that the epididymal duct (mesonephric duct) was divided into caput, corpus and cauda at 130 days of gestation.

Mohamed (2005) observed that the epididymal duct appeared as mesonephric duct in bovine foetii at CRL 10 -13 cm and slight coiling was found in the head region at 18 – 20 cm CRL when the body and tail was straight. The coiling of the epididymal duct started in cauda region at 24 cm CRL and in body at 30 – 60 cm CRL in bovine foetus. He also observed that caput, corpus and cauda part of epididymis were differentiated at 24 cm CRL.

Inomata et al. (2009) observed the presence of Wolfian duct at the ventro – lateral side of mesonephros in 0.8 cm CRL cat foetuses. The cranial tip of Wolfian duct was located at the cranial end of the mesonephros, caudal to the attachment of mesonephros diaphragmatic ligament. At 7.0 cm CRL, the Wolfian duct began to develop into the epididymal duct and required androgens to maintain its development.
b. Epithelial differentiation

Moustafa and Hafez (1971) noticed that in the caput region the epididymal duct was lined by tall columnar cells with stereocilia and their apical cytoplasm contained secretory granules and droplets, which were first evident at 150 days of gestation in bovine foetus.

Russe and Sinowatz (1991) noticed that initially the epithelial lining of the epididymal duct was simple cuboidal to low columnar, which differentiated later on into the characteristic tall columnar cells with stereocilia in bovine foetuses.

Mohamed (2005) observed that the mesonephric duct was lined by a simple cuboidal or low columnar epithelium having large oval nuclei, one or more nucleoli with well distinct basement membrane in bovine foetus of 74 to 87 days of gestation. At 109 to 118 days of gestation the epididymal duct was lined by simple cuboidal or low columnar epithelium equipped with stereocilia which transformed into simple columnar epithelium with stereocilia afterwards.

In cat foetus the Wolfian duct was lined with a layer of cuboidal epithelial cells at 0.8 cm CRL (Inomata et al. 2009).

c. Peritubular tissue development

Mohamed (2005) reported 7 – 15 layers of peritubular mesenchymal cells in bovine foetal epididymis at 74 - 87 days of gestation which became 7 layered at 109 – 118 days of gestation and again increased to 10 – 15 layers at 128 days of embryonic life when most of the peritubular mesenchymal cells had transformed into smooth muscle cells. During further development, the number of peritubular smooth muscle cell layer was different in different regions of epididymis and it was observed that the number of these layers increased from caput to cauda. At last month of gestation the number of peritubular smooth muscle cell layer was about 5 in caput and corpus and about 8 – 10 in cauda.

A thin layer of smooth muscle cells surrounded the epithelium of entire epididymal duct in bovine foetus and the thickness of these layers increased distally (Mustafa and Hafez 1971 and Russe & Sinowatz 1991).

The peritubular tissue comprising of differentiating smooth muscle cells was observed in the epididymis of day old buffalo calf which was found to be more compact and better differentiated at 2 – 2.5 month of age (Singh 1989).

d. Capsule and stroma

Riar et al. (1973) noticed that the intertubular stroma was minimum in caput and increased in the cauda of mammalian epididymis.
Mohamed (2005) demonstrated that interstitium was highly cellular and vascular with the large mesenchymal cells in the bovine epididymis of 10 – 13 cm CRL. With the increase in the age, these cells also increased in size with large round vesicular nuclei and large cytoplasmic processes. At the end of gestation (CRL 90 cm) the interstitium contained differentiating fibroblast and different type of leukocytes along with connective tissue fibres.

Jorma et al. (1986) revealed the presence of mesenchymal area with few collagen fibres around the genital ducts of 15 day old male rat foetus. They further observed that the amount of collagen fibrils increased with the histological maturation of the mesenchyme around the mesonephric duct.

e. Micrometry

The foetal epididymis of rhesus monkey composed of many small round ducts at 45 days and the diameter of these ducts increased at 90 days. They maintained their size until 120 – 150 days of gestation and at 150 days the ductal diameter again increased to 110 mm (Alexander, 1972).

Arrighi and Domeneghini (1993) found few tubular profiles of 92 ± 20 µm in diameter and of epithelial height 29 ± 4 µm in ductus epididymis of cat foetii at 30 – 40 days of gestational age.

De Miguel et al. (1998) stated that the development of the ductus epididymidis in human followed a biphasic pattern similar to efferent ductules. A progressive increase in total tubular surface, total luminal surface, tubular diameter, luminal diameter and epithelial height occurred from foetal period to 2.4 months of age. However, this development was transient and regressed during infancy. At childhood, the development started again and completed at puberty.

2.3. Histochemistry
2.3.1. Testes
(i) Carbohydrates

a. Periodic acid Schiff (PAS) reaction

Carbohydrates serve as an endogenous source of energy. Johnson et al. (1970) observed positive reaction for PAS in primordial germ cells of genital ridge in domestic animals and negative reaction in the mesenchymal cells. Guraya (1974 a, b) and Kerr & Knell (1988) reported the presence of glycogen in the man, guinea pig and rat foetal Leydig cells respectively. Kanai et al. (1989) and Pyne & Sinha (1989) reported glycoconjugates
on the somatic cell surface and in the small dense bodies of germ cells in mice and goat embryo, respectively. Fazel et al. (1987) and Malmi et al. (1990) reported distribution of glycoconjugates during development of testis of rat. Frojdman et al. (1992) reported the presence of abundance of carbohydrates in the early foetal gonads of rat.

Nagano et al. (1999) reported the distribution of sugar residues in cytoplasm and plasma membrane of gonocytes in the differentiating mouse testis on 16.5 days post coitus. Similar findings were observed in the testes of mouse by Davies et al. (1975).

Kaur et al. (2008) observed a weak PAS positive reaction in germinal epithelium, tunica albuginea (both fibrosa and vasculosa layers) and mediastinum testis in the buffalo foeti between 10 cm – 18.2 cm CRL. At CRL 21.6 cm – 39.5 cm, similar reaction was observed in tunica albuginea and moderate reaction in mediastinum testis however, a moderate to strong reaction was observed in the germinal epithelium and seminiferous tubules at this stage of gestation. They further stated that with the advancement of the age basement membrane of seminiferous tubules showed an increasing pattern (moderate to strong) of PAS activity.

In buffalo foeti of 75.0 cm CRL a strong PAS positive reaction was observed in the cytoplasm of gonocytes where as weak reaction was observed in the cytoplasm of pre – sertoli cells (Kaur et al. 2008). They reported a weak PAS activity in the interstitial tissue at CRL – 10 to 18.2 cm and stated that it increased to moderate at CRL – 21.6 cm onwards in buffalo foeti.

Farooqui et al. (2012) observed that the PAS activity at 31 – 60 days of gestation in goats was intense in the basement membrane of germinal epithelial cells, mild to moderate in the connective tissue of septula testis and moderate in the large and small cells. At the foetal age of 61 – 90 days the reaction for PAS was moderate in large cells and mild to moderate in small cells, however, at 91 – 120 days of gestation the activity was mild to moderate in large and small cells.

An intense reaction for PAS in the cytoplasm of the cells of tunica vaginalis visceralis was observed at 91 – 120 days of gestation in goats. Endothelium of blood vessels in the tunica vasculosa showed an intense PAS reaction at the end stage of gestation. At 116th day of gestation and onwards, basement membrane of convoluted sex cords and rete tubules was highly acidophilic and exhibited moderate to intense PAS reaction (Farooqui et al. 2012).

Farooqui et al. (2012) observed a moderate reaction for PAS in the supra nuclear zone of sertoli cells and an intense reaction in the basement membrane of straight tubules
in goat foetal testes at the age of 61 days and onwards. They also observed a moderate PAS activity in the interstitial space at 44\textsuperscript{th} day of gestation and onwards. A very weak reaction was observed in foetal leydig cells.

In goat foetus contents of the interstitial space showed moderate reaction for PAS and cytoplasm of the sertoli cells showed moderate to intense reaction for PAS in supranuclear zone (Farooqui et al. 2012). However, in buffalo foeti weak and weak to moderate PAS reaction was observed in interstitial tissue and pre – sertoli cells respectively by Kaur et al. (2008).

b. Acidic mucopolysaccharides (Alcian blue reaction)

Guraya (1980) stated that intertubular tissue was devoid of AMPS (acid mucopolysaccharides) in the foetal testes, which might be due to the presence of steroidgenic enzymes in the interstitium of foetal testes. Silberstein and Daniel (1984) stated that the weak to moderate alcinophilia may be responsible for glycosaminoglycans synthesis, degradation and morphogenic activities in the specific areas.

Kaur et al. (2008) in buffalo foeti observed a weak alcian blue positive reaction at CRL 10 cm to 39.5 cm in tunica albuginea, seminiferous tubules, basement membrane and mediastinum testis, however, moderate to strong reaction was observed at CRL 44 cm onwards.

In buffalo foetal testis at CRL 10 cm - 18.2 cm, no activity of acid mucopolysaccharides was observed in germinal epithelium and at CRL 21.6 cm – 39.5 cm weak to moderate and at CRL 44 cm onwards strong reaction was observed for acid mucopolysaccharides in germinal epithelium by Kaur et al. (2008). They further stated that the intertubular tissue was devoid of AMPS in the buffalo foetal testes.

Farooqui et al. (2012) reported that at 31 – 60 days of foetal age in goats’ cytoplasm and basement membrane of germinal epithelial cells showed moderate reaction for acid mucopolysaccharides. They reported intense reaction for acid mucopolysaccharides in the cytoplasm of the cells of tunica vaginalis visceralis at 91 – 120 days of foetal age.

In goats primordial germ cells (large cells) showed moderate reaction for acid mucopolysaccharides throughout gestation. In mesenchymal cells (small cells) intense reaction was seen at the age of 31 – 60 days, moderate reaction at 61 – 90 days of age, moderate to intense at 91 – 120 days and weak reaction at 121 days onwards (Farooqui et al. 2012).
In the connective tissue of septula testis a moderate reaction for acid mucopolysaccharides was observed by Farooqui et al. (2012) in goat foetal testis at the age of 44 days and onwards. At 61 – 90 days of age cytoplasm of cells of rete tubules exhibited moderate activity for acid mucopolysaccharides.

Farooqui et al. (2012) reported intense reaction at 31 – 90 days and moderate reaction at 91 days onwards for acid mucopolysaccharides in supra nuclear zone of the sertoli cells. They observed moderate to intense reaction in the interstitial space of goat foetal testis. In interstitial endocrine cells moderate to intense reaction was observed at 31 – 90 days of gestation and moderate activity till term.

(ii) Lipids

The supporting cells showed the presence of few lipid droplets in the guinea pig (Black & Christensen 1969), man (Pelliniemi 1970) and rabbit (Gondos and Conner 1973 and Bjerregaard et al. 1974). Merchant (1975) and Pelliniemi (1975b) also made similar observations in rat foetus and both male and female gonads of pig foetus, respectively.

Gondos et al. (1974) observed prominent lipid droplets in leydig cells between 12 – 13 days of gestation in hamster foetal testis. Davies et al. (1975) reported two lipid fractions in the interstitial cells of horse foetus. In Macaca fascicularis, foetal leydig cells contained 19% SER and 5% lipid droplets after differentiation phase but after involution phase the SER decreased to 12% where as lipids doubled (Fouquet et al. 1978). Kerr and Knell (1989) and Guraya (1974 a, b) reported the presence of lipid inclusions in the rat and man foetal leydig cells respectively.

Lipids form structural components of membranes and help in storage and transport of metabolic fuel (Lehninger 1975).

Guraya and Uppal (1977) reported the presence of diffuse lipoproteins and a few lipid granules consisting of phospholipids in the foetal testes of field rat. Wartenberg (1978 & 1983) demonstrated the presence of few lipid droplets in the supporting cells of human foetus. Van Vorstenbosch et al. (1984) reported the presence of a large lipid droplet beside the nucleus of the sertoli cells until 52 days post coitum in pig foetus.

Kaur et al. (2008) reported negligible reaction for sudanophilic lipids in germinal epithelium, basement membrane and tunica albuginea till CRL 39.5 cm, however, at CRL 44 cm onwards they observed moderate to strong reaction in tunica vasculosa, weak reaction in pre sertoli cells and mild to moderate reaction in gonocytes in buffalo foetii. They also stated that diffuse sudanophilic reaction in leydig cells at CRL 44 to 90 cm in
buffalo foeti might be due to hypertrophy of foetal leydig cells which became active during foetal life and were responsible for steroid production.

Farooqui et al. (2012) stated that the moderate sudanophilic reaction was observed after 61 days of gestation in the tunica albuginea of goat foetal testis. The vascular layer showed relatively less number of lipid globules as compared to tunica fibrosa at 121 days of gestation. In large and small cells they observed mild activity for lipids throughout gestation. In sertoli cells of goat foeti, no activity was recorded till 60 days of gestation however a weak reaction was observed 61 days onwards.

Farooqui et al. (2012) observed that the lipids were present in mild concentration in the interstitial space only at 91 – 120 days of gestation and mild to moderate concentration was recorded in the leydig cells at 90 days of age and onwards.

(iii) Proteins

Courot et al. (1970) and Pelliniemi et al. (1993b) reported that the establishment of spermatogenesis was indicative of protein synthesis required for structural and metabolic needs of proliferating and differentiating sertoli cells.

The cytological features of mammalian embryonic sertoli cells have all organelles which were indicative of protein synthesis (Gondos 1977 and Guraya 1980).

In rat foetal testis the leydig cells had highest steroid concentration at 18.5 day of gestation and there was a sharp decline during the last foetal days (Tapanainen et al. 1984).

Pelliniemi et al. (1984) and Paranko (1987) reported distribution of cytoskeletal proteins and glycoproteins in and around the epithelial gonadal cords during sexual differentiation in human and rat, respectively.

Rabinovici & Jaffe (1990) observed presence of proteins in the pre sertoli cells of primates.

Majdic et al. (1997) reported the expression of proteins in rat foetal sertoli and leydig cells at day 14.5 post coitum but reported no evidence in the germ cell.

Kaur et al. (2008) observed weak reaction for proteins in germinal epithelium, tunica albuginea, mediastinum testis, basement membrane and seminiferous tubules in the buffalo foeti of 10 cm – 18.2 cm CRL. At CRL 21.6 – 39.5 cm strong reaction in germinal epithelium, moderate to strong in tunica albuginea and mediastinum testis, moderate reaction in basement membrane and weak reaction in seminiferous tubules was observed. At CRL 44 cm onwards they observed strong reaction in germinal epithelium, tunica albuginea and connective tissue of mediastinum testis, moderate reaction in basement membrane and seminiferous tubules, moderate to strong reaction in gonocytes and channels of rete testis.
2.3.2. Epididymis

(i) Ductuli efferentes

a. Carbohydrates

Pelliniemi et al. (1983) reported small glycogen particles in the proximal mesonephric tubules and the primordial of the ductuli efferentes in human embryo.

Goyal and Williams (1988) noticed a PAS positive reaction in the type II non-ciliated cells and no reaction in type III cells in ductuli efferentes of goat.

Pal and Bhardwaj (1989) found a moderate PAS reaction in the granulated and vacuolated cytoplasm of basal cells in prepubertal animals while mild PAS reaction was observed in the pubertal and post pubertal buffalo bulls.

Khurana (2000) demonstrated the neutral mucopolysaccharides in the ductuli efferentes of bull and found a strong PAS reaction in apical portion of epithelial cells, cilia and basement membrane in prepubertal and adult animals.

A strong PAS positive reaction indicating the presence of neutral mucopolysaccharides was observed in basement membrane of efferent ductules in bovine foetuses (Mohammad 2005) and in buffalo foetus (Bansal and Uppal 2009).

Sharma (2010) observed weak to moderate PAS positive reaction in the apical portion of efferent ductules of 0 – 20 cm CRL buffalo foeti. In 20 – 40 cm CRL foetus moderate to strong PAS activity was observed in apical portion of epithelial cells and basement membrane. He further reported that in buffalo foeti of CRL above 40 cm a weak reaction was observed in connective tissue components of stroma, weak to moderate in capsule, moderate to strong PAS reaction in supranuclear part of epithelial cells & cilia and a strong PAS positive activity was noticed in basement membrane surrounding the epithelium.

b. Acidic mucopolysaccharides

Khurana (2000) demonstrated the acid mucopolysaccharides in the ductuli efferentes of buffalo bull and observed a moderate alcian blue (AB) reaction in the supranuclear part of epithelial cells, cilia and basement membrane in prepubertal animals and weak reaction of alcian blue in the pubertal and adult animals.

In buffalo foeti of CRL 0 – 20 cm a weak reaction for acid mucopolysaccharides was observed in coelomic epithelium by Sharma (2010). In 20 – 40 cm CRL buffalo foeti weak to moderate activity of acid mucopolysaccharides was noticed in capsule and stroma. He further reported a weak activity in connective tissue component of stroma and weak to
moderate activity in capsule and supranuclear part of epithelial cells and cilia in buffalo foeti of CRL 40 cm and above.

c. Lipids

Khurana (2000) observed a moderate to strong intensity of sudanophilic lipids in the supranuclear part of epithelial cells in prepubertal period.

Sharma (2010) observed a weak reaction for sudanophilic lipids in tunica albuginea and intertubular stroma in buffalo foetal epididymis. The epithelial lining showed weak to moderate reaction for sudanophilic lipids. He further stated that accumulation of lipid droplets in ductular epithelial cells was more towards the luminal border and these droplets were observed in buffalo foeti throughout gestation.

d. Proteins

In 0 – 20 cm CRL and 20 – 40 cm CRL buffalo foeti Sharma (2010) observed a weak reaction for basic proteins in the capsule of efferent ductules. The apical and supranuclear part of epithelial cells had weak activity in 0 – 20 cm CRL buffalo foeti and CRL 20 cm onwards a weak to moderate activity was observed. The peritubular connective tissue showed a moderate reaction where as the intertubular stroma had a very weak reaction in buffalo foeti of CRL 20 – 40 cm and onwards (Sharma 2010)

(ii) Ductus epididymis

a. Carbohydrates

Neutral mucopolysaccharides

Goyal and Dhingra (1975) found intense PAS positive reaction in the fibrous capsule and moderate reaction in intertubular connective tissue of buffalo epididymis. The apical region of epithelial cells contained numerous PAS positive granules at 3 – 52 weeks of age which decreased in number in 72 – 76 weeks. They also detected a weak PAS positive activity in the stereocilia.

Zondek and Zondek (1980) noticed that the foetal epididymis had a weak PAS reaction by 25th weak of gestation which gradually increased and reached to its peak in the later weeks of pregnancy in human foetus and then decreased after the 3rd month of life. It can be assumed that androgens produced by the foetus in response to maternal and placental hormones were responsible for the secretions of the epididymis during foetal life.

Pelliniemi et al. (1983) observed that the epithelial cells of the human mesonephric duct contained large clear areas in the intranuclear cytoplasm and showed a PAS positive reaction indicating the presence of glycogen. The glycogen accumulation was most
prominent between the ages of 5 to 10 weeks of prenatal development and disappeared by the age of 15 weeks.

Pal and Bhardwaj (1986) reported a mild PAS reaction in columnar cells and intermediate in the basal cells at prepubertal age in buffalo. Singh (1989) described in buffalo calf epididymis that tunica albuginea, septa, stroma and peritubular smooth muscle cells were weakly PAS positive where as epithelium showed a weak to moderate reaction for PAS.

Mohammad (2005) observed a distinct PAS positive reaction in the basement membrane underneath the epithelium of epididymis of bovine foetus. Sharma (2010) reported that in buffalo foetii the tunica albuginea showed weak to moderate reaction for neutral mucopolysaccharides in the caput and corpus and weak activity in the cauda epididymis. A strong PAS positive reaction was observed in the basement membrane and in the apical portion of the epididymal epithelium in the caput, corpus and cauda of buffalo foetii of all the age groups.

**Acidic mucopolysaccharides**

Goyal and Dhinigrai (1975) found moderate AB reaction in stereocilia. He further stated that the epithelium was weakly reactive to AB in all age groups of buffalo foetii. Singh (1989) reported in buffalo calf epididymis that epithelium showed a weak reaction for acid mucopolysaccharides. Sharma (2010) observed a weak to moderate reaction for acid mucopolysaccharides in the tunica albuginea of caput and corpus regions and weak activity in the cauda region of the epididymis of buffalo foetus. The epithelium was found to be weakly reactive to AB in all age groups of buffalo foeti. Stereocilia showed moderate reaction for acid mucopolysaccharides.

Weak activity for acid mucopolysaccharides was observed in the peritubular area and stroma of 20 – 40 cm CRL buffalo foeti; where as a weak to moderate reaction was observed in the stroma of buffalo foeti above 40 cm CRL in all the regions of epididymis (Sharma 2010).

**b. Lipids**

Singh and Dhinigrai (1971) reported the presence of lipid droplets in the apical part of columnar cells and supranuclear part of basal cells in epididymis of buffalo calf. The lipids could not be demonstrated in capsule and intertubular tissue except endothelial lining of blood vessel in buffalo (Goyal and Dhinigrai 1975). They also noticed more sudanophilic lipid in tail region than the head and body of epididymis.
Pal and Bhardwaj (1986) investigated that lipids were more in epididymal epithelium of pubertal buffalo bull than in prepubertal and postpubertal animals. Singh (1989) observed fine lipid droplets of weak to moderate intensity in basement membrane and luminal border of epithelium and stereocilia in buffalo calf.

Sharma (2010) observed a weak reaction for sudanophilic lipids in tunica albuginea; inter tubular stroma and peritubular tissue and weak to moderate reaction in basement membrane, luminal border of epithelium and stereocilia in buffalo foetii. He stated that no regional variation was observed in the demonstration of lipid droplets in the ductus epididymis of buffalo foetii in relation to foetal age.

c. Proteins

Singh (1989) demonstrated a moderate reaction for basic proteins in the epididymal epithelium of head region of buffalo epididymis which increased to moderate to strong in body and tail regions whereas peritubular tissue showed moderate reaction for basic proteins in all the regions of epididymal duct at 5 – 12 month of age.

Sharma (2010) found a weak reaction for basic proteins in supranuclear part of epididymal epithelium of 12.5 cm CRL buffalo foeti which increased to moderate in 20 – 40 cm CRL buffalo foeti and moderate to strong in buffalo foeti of CRL 40 cm onwards. He further stated that weak to moderate activity of basic proteins was observed in stroma of caput, corpus and cauda of epididymis in buffalo foeti at CRL 40 cm and onwards. Moderate activity of basic proteins was observed in the stereocilia and peritubular tissue in the cauda region of epididymis in buffalo foeti of CRL 40 cm onwards.

2.4. Mineral, biochemical and enzyme studies in foetal fluid

Wahid et al. (1991) reported that foetal membranes were extra embryonic in nature and were developed for the purpose of physiological exchanges between foetal and maternal tissues. Dynamic system of foetoplacental unit caused constant exchange of water and fluid between foetal compartments and maternal circulation, which was reflected in changes in the physical, chemical and biochemical constituents of foetal fluids (Aidasani et al. 1993). Foetal fluids were important in the efficient handling of foetal waste products and in preventing mechanical shock to the developing foetus during entire gestation (Amle et al. 1992).

The concentration of constituents in amniotic and allantoic compartments was influenced by exchange through the placenta, metabolic products of the foetus, foetal urine formation and fluid flow through the urachus or urethra and foetal secretions from lung and salivary glands. Urine from the bladder of the foetus could pass through the
urachus into the allantoic sac or through the urethra into the amniotic sac (Baetz et al. 1976; Javed 1990 and Foulds et al. 1998). However, both amniotic and allantoic fluids differed substantially in composition than that of foetal urine (Javed 1990). The study of biochemical profiles in foetal fluids can be used as a tool for pregnancy diagnosis and for knowing the status of the growing foetus. Therefore, understanding the normal values would be a useful index in the determination of the physiological aspects in non pregnant or pregnant ewes. These indices may vary depending on factors such as sex, age, weather, stress, season and physical exercise (Kaneko et al. 1999).

2.4.1. Allantoic fluid

The allantoic sac was considered mainly as a reservoir for foetal wastes, however recent investigations with pigs have shown that allantoic sac plays an important role in the accumulation of nutrients and metabolism of some factors like uteroferrin and iron, suggesting a hitherto unrecognized function of the allantoic sac in foetal nutrition (Kwon et al. 2004).

Glucose from the maternal circulation is the main energy source for the foetus during normal pregnancy. Part of this glucose is reduced to sorbitol in the placenta. The sorbitol is then oxidized to fructose in the placenta or the foetal liver. Fructose is not used by the foetus unless the flow of glucose from the mother is interrupted (Baetz et al. 1976 and Javed 1990).

Khatun et al. (2011) observed a decrease in the level of glucose (3.57 ± 0.34 to 2.30 ± 0.43 mg %) as the gestation period advanced (14 - 57 days to 121 to 140 days) but the changes were statistically non significant in sheep at different stages of development. Khojasteh et al. (2011) in goat (10.3 ± 14.2 to 4.2 ± 4.6 mg/dl), Mufti (1995) in sheep and Baetz et al. (1976) in bovine also recorded a decrease in glucose concentration from early to late stages of gestation.

McDonald (1980) reported that increased protein concentration in maternal circulation indicates a positive association of pregnancy establishment and clearance of redundant protein from this compartment was possibly more restricted and dependent on the proteolytic breakdown of proteins to smaller polypeptides and amino acids prior to removal (Riding et al. 2008). Khatun et al. (2011) reported an increase in total protein concentration from 0.48 ± 0.06 g/dl to 1.42 ± 0.20 g/dl at 14 – 57 days to 121 - 140 days of gestation in sheep. Khojasteh et al. (2011) stated that the protein concentration was 97.80 ± 149.4 mg/dl at 30 – 60 days of gestation in goat which increased to 128.9 ± 128.2 mg/dl at 90 – 120 days of gestation and then at 121 days and till term it decreased to 85 ±
A gradual increase in the concentration of total proteins during gestation period has also been reported earlier in domestic animals (Kaneko and Corneleous 1970), cattle (McDonald 1980), sheep (Mufti 1995) and goats (Lathura et al. 1987).

In sheep as the gestation advanced increase in the mean concentration of urea (16.60 ± 1.02 mg% to 72.36 ± 1.08 mg %), urea nitrogen (7.75 ± 0.48 to 33.79 ± 0.50 mg %) and creatinine (2.21 ± 0.37 mg % to 16.77 ± 0.31 mg %) was observed from 14 to 140 days of gestation (Khatun et al. 2011). Khojasteh et al. (2011) reported that creatinine and uric acid are the constituents that could change by increasing their concentration with gestational age in the allantoic fluid of goat however they observed a decrease in the concentration of urea as gestation advanced. Mellor et al. (1975) reported that urea concentration in bladder of sheep foetus increased during pregnancy. According to Tangalakis et al. (1995), until day 75 of pregnancy in sheep, foetal urine drains exclusively into the allantoic sac, while in the final third of gestation, it drains equally into both amniotic and allantoic sacs. So they concluded that decrease in urea concentration in last stages of gestation might be attributed to entrance of some urine into amniotic sac. Baetz et al. (1976) and Alexander et al. (1958) had observed an increasing trend in the concentration of creatinine in bovine and in sheep respectively during gestation period. Stainer (1965) explained that lack of exchange of solutes from allantoic fluid might be the cause of increased level of creatinine in pregnant animals.

Khatun et al. (2011) observed no significant difference in the level of cholesterol (3.13 ± 0.19 mg %) during different stages of gestation in sheep. Mufti (1995) stated that the level of cholesterol remained constant in foetal fluids serving need for synthesis of progesterone during entire gestation length in sheep.

Khatun et al. (2011) stated that the level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased from 6.34 ± 0.24 U/L to 5.62 ± 0.82 U/L and 13.48 ± 0.42 to 12.43 ± 0.50 U/L respectively at 14 to 93 days of gestation and then increased upto 140 days of gestation in allantoic fluid of sheep. The mean concentration of alkaline phosphatase (ALP) (27.33 ± 1.42 KA units to 36.50 ± 1.26 KA units) increased as the gestation advanced from 14 to 140 days.

Khojasteh et al. (2011) observed that the concentration of sodium in allantoic fluid of goat increased from 0 – 30 days (75.3 ± 51.2 mmol/L) to 30 – 60 days (77.80 ± 39.2 mmol/L) of gestation and then decreased from 60 to 120 days (75.3 ± 39.2 mmol/L to 73.5 ± 38.6 mmol/L) and was maximum at 121 days till term (78.9 ± 67.2 mmol/L). The level of chloride and phosphorus was found to be decreased from 0 – 30 days (70 ± 82.6
mmol/L and 5.1 ± 4.4 mg/dl, respectively) to 121 days and till term (30.2 ± 42.2 mmol/L and 4.1 ± 14.6 mg/dl respectively) while the level of potassium was increased from 5.00 ± 2.6 mmol/L to 5.80 ± 3.8 mmol/L in allantoic fluid of goat at 0 – 30 days to full term of gestation.

Aidasani et al. (1993) reported that the concentration of sodium, potassium, magnesium and phosphorus was 18.72 ± 2.01 mEq/L, 20.72 ± 4.80 mEq/L, 7.68 ± 0.90 mEq/L and 2.77 ± 0.38 mg/100ml respectively in the allantoic fluid of goat.

### 2.4.2. Amniotic fluid

In cattle and sheep, formation of amnion occurs on 13 – 16 days of pregnancy and then amniotic fluid fills the amniotic sac (Robert 1986) and surrounds the developing embryo. A broad knowledge of amniotic fluid is of the utmost importance in understanding foetal metabolism and identifying pathologic conditions during pregnancy (Prestes et al. 2001).

Changes in the foetal fluid had an orientation of foetal adrenocortical activity towards glucose homeostasis. Until about 130 days of gestational age in sheep, exchange of glucose occurred between the amniotic sac and foetal blood (Mellor and Slater 1974). Elevated levels of glucose in the amniotic fluid might be associated with concurrent cortisol secretion by the foetus (Williams et al. 1993). In normal pregnancy, amniotic fluid glucose levels correlated with simultaneous maternal glucose concentrations (Archimaut et al. 1974) and rose acutely in response to maternal glucose load (Greco et al. 1980).

Khatun (2011) stated that the glucose concentration in amniotic fluid decreased from 4.33 ± 0.42 mg % to 2.92 ± 0.15 mg % in sheep at 14 – 57 days to 121 – 140 days of gestation. Khadjeh et al. (2007) also reported a decrease in glucose concentration in goat from 0 – 30 days (7.1 ± 4.5 mg/dl) of gestation to 121 days till term (3.6 ± 3.3 mg/dl). Similarly, Prestes et al. (2001), Bradly and Misrette (1973) and Reddy et al. (1995) recorded a decrease in glucose concentration in sheep amniotic fluid. Prestes et al. (2001) explained that decrease of glucose in amniotic fluid during gestation might be due to foetal intake of glucose as a consequence of the foetal swallowing reflex. However, Anitha and Thangavel (2011) stated that the glucose concentration increased from 17.09 ± 3.52 mg/dl at 30 – 60 days of gestation to 57.29 ± 4.31 mg/dl at 121 days and till term in the amniotic fluid of goat, similarly Aidasani et al. (1992) and Tungalakis et al. (1995) reported an increase in glucose concentration in goat and sheep amniotic fluid, respectively during gestation period although the last two authors used different methods to measure glucose at different stages of pregnancy.
Amniotic fluid proteins were apparently derived from both maternal and foetal sources. The maternal contribution was principally the globulin fraction, while the foetus contributed prealbumin, albumin, fetoprotein and sex hormone binding globulin. Passage of proteins across the amnion might have occurred in either direction; the net quantity remaining in amniotic fluid also bears upon apparent concentration (Dito 1970). The foetus synthesized all its proteins from the amino acids derived from the mother and they were used mainly for synthesis rather than oxidation or gluconeogenesis (Jainudeen and Hafez 1980).

Khatun et al. (2011) stated that the average concentration of total proteins in the sheep amniotic fluid increased from 0.40 ± 0.06 g/dl at 14 – 57 days to 0.84 ± 0.26 g/dl at 121 – 140 days of gestation. Anitha and Thangavel (2011) also reported increase in the concentration of total proteins from 0.51 ± 0.08 mg/dl to 1.17 ± 0.08 mg/dl in sheep amniotic fluid at 30 – 60 days to 121 days of gestation and till term. Khadjeh et al. (2007) reported maximum concentration of total proteins to be 0.54 ± 0.9 g/dl in amniotic fluid of goat at 0 – 30 days of gestation with subsequent decrease in later stages of gestation (30 – 60 days to 121 to term). Reddy et al. (1995) reported that the low concentrations of total protein in amniotic fluid could be attributed to the absence of fibrinogen and other proteins due to foetal liver immaturity.

According to Mellor and Slater (1972 and 1973) and Mellor et al. (1975), urea concentration in foetal bladder was increased during pregnancy. Wintour et al. (1986) stated that in mid gestation, foetal urine, high in urea and low in chloride entered the amniotic fluid, which resulted in an increase in amniotic fluid urea and decrease in chloride. Khatun et al. (2011) reported an increase in the mean concentration of urea (14.01 ± 0.72 mg % to 38.10 ± 0.47 mg %), urea nitrogen (6.54 ± 0.34 to 17.79 ± 0.22 mg %) and creatinine (1.52 ± 0.26 mg % to 13.67 ± 0.75 mg %) from 14 to 140 days of gestation in sheep. Khadjeh et al. (2007) observed an increase in the mean concentration of urea (43.7 ± 30.3 mg/dl to 91.70 ± 101.3 mg/dl), uric acid (0.40 ± 0.6 mg/dl to 0.70 ± 0.3 mg/dl) and creatinine (1.50 ± 1.9 mg/dl to 5.90 ± 4.6 mg/dl) in the amniotic fluid of goat as the gestation advanced. Prestes et al. (2001) also reported an increase in the mean concentration of uric acid and creatinine but a decrease in the concentration of urea in sheep as the gestation advanced. Lovell et al. (1995) and Aidasani et al. (1992) recorded low creatinine levels in the amniotic fluid of goat at the beginning of pregnancy.

Khadjeh et al. (2007) found that sodium (123.3 ± 10.9 mmol/L) and potassium (6.2 ± 1.6 mmol/L) levels were highest at 30 – 60 days and 0 – 30 days of gestation,
respectively and then decreased towards the term. Prestes et al. (2001) through amniocentesis on 70, 100 and 145 days of pregnancy in sheep, reported that sodium and potassium values decreased from 70 to 145 days of pregnancy. They further stated that the mineralocorticoids activity of intrauterine foetal maturity acts on foetal kidneys, increasing potassium and decreasing sodium concentrations in foetal urine.

According to Wintour et al. (1986) a classical sodium pump may be responsible and alterations in the relative permeability to sodium and potassium may affect the transport.

Khadjeh et al. (2007) stated that the level of chloride in amniotic fluid increased from 101.4 ± 31.1 mmol/L to 117.3 ± 17.8 mmol/L in goat at 0 – 30 days to 60 – 90 days of gestation and then decreased towards term.

Khatun et al. (2011) stated that the level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased from 7.56 ± 0.24 U/L to 6.98 ± 0.76 U/L and 15.26 ± 0.45 U/L to 13.40 ± 0.40 U/L respectively at 14 to 93 days of gestation and then increased up to 140 days of gestation in amniotic fluid of sheep. The mean concentration of alkaline phosphatase (ALP) (30.73 ± 1.51 KA units to 43.75 ± 1.31 KA units) increased as the gestation advanced from 14 to 140 days.

Aidasani et al. (1993) reported that the concentration of sodium, potassium, magnesium and phosphorus was 37.24 ± 3.89 mEq/L, 4.64 ± 0.86 mEq/L, 2.53 ± 0.24 mEq/L and 4.31 ± 1.31 mg/100ml respectively in the allantoic fluid of goat.