STUDY ON HISTOGENESIS OF KIDNEY IN SHEEP (Ovis aries)

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I.D No: GVM/2014-051

Thesis submitted to the

SRI VENKATESWARA VETERINARY UNIVERSITY

in partial fulfilment of the requirements
for the award of the degree of

MASTER OF VETERINARY SCIENCE

In the faculty of Veterinary Science

(VETERINARY ANATOMY)

DEPARTMENT OF VETERINARY ANATOMY

NTR COLLEGE OF VETERINARY SCIENCE, GANNAVARAM – 521 102

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AUGUST-2017

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No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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ACKNOWLEDGEMENTS

I thank "The Almighty" for giving me the strength to keep going. I thank you for being the best listener and blessings of my family to complete this task.

First and foremost, I express my sincere and deepest thanks to Chairman of my advisory ***Dr. (Mrs.) Yamani Nagamalleswari, Professor, Department of Veterinary Anatomy, NTR College of Veterinary Science, Gannavaram****, for her personal attention, persuasion, constant guidance and encouragement, and for her moral support for filling me with high levels of positive energy during the research work. Thank you for bearing with me despite my flaws. My sincere gratitude for great patience and calmness. I owe her for co-operation, motivation and support have always kept me going ahead.*

My special words of thanks to my research co-guide ***Dr. N.K.B. Raju, Associate Professor, Department of Veterinary Anatomy, NTR College of Veterinary Science, Gannavaram,*** *for his support, guidance, cooperation and encouragement. I owe a lot of gratitude to him and I Feel privileged to be associated with a peaceful person like him during my life.*

I indebted my deepest gratitude to ***Dr. M. Raghunath, Associate Professor, Department of Veterinary Surgery & Radiology, NTR College of Veterinary Science, Gannavaram,*** *for his kind concern and support.*

I extend my thanks to ***Dr. P. V. S. Kishore, Professor and Head, Dept. of Veterinary Anatomy, NTR College of Veterinary Science, Gannavaram*** *for support, patience and steadfast encouragement for every aspect to complete this study.*

I express my sincere thanks to ***Dr. Jagapathi Ramayya, Professor and Head, Dept. of Veterinary Anatomy, College of Veterinary Science, Tirupati*** *for his, valuable suggestions.*

I cordially put forth my acknowledgements to ***Dr. Archana, Dr. Raja*** *and* ***Dr. Chandana, Assistant Professors, Department of Veterinary Anatomy, NTR College of Veterinary Science, Gannavaram,*** *for their encouragement during the research work.*

I express my deepest gratitude to ***Dr. P. Kalyani, Assistant Professor, Department of Biochemistry,*** *for her valuable suggestions and support during thesis writing.*

I express my deep sense of thanks to ***Dr. Naveen Swaroop, Assistant Professor, Department of Biochemistry,*** *who was very much helpful in utilizing cryostat facility.*

I sincerely put forth my acknowledgements to ***Dr. Muralidhar, Assistant Professor, Department of Animal Genetics & Breeding,*** *for his help in statistical analysis.*

I would like to acknowledge ***Dr. T Srinivas Rao, Associate Professor & Head, Department of Veterinary Public Health,*** *for enabling me to utilize microscope facility.*

Nothing will be sufficient to express my deep sense of obligation to my beloved seniors ***Dr. Karunasri, Dr. Dhyana, and Dr. Nagendra*** *for their nice company and cooperation.*

Memories of my life are attached and were gifted through the friendship that was built here with my colleagues ***M. Basavaiah, N.Swetha*** *for their guidance and moral support.*

I am in short of words to express my love and gratitude to my dear juniors M.V.Sc., ***Tharun*** *and* ***Swetha*** *for their help during my study.*

Nostalgia is like a grammar lesson. You find the present tense but the past perfect. I express my thanks from bottom of my heart to my comrade friends ***Ekonthung, Charujith, Rajsekhar, Sairam, khalif, Morothung, Kaushlendra, Najeeb, Radha, Deepak, Varaprasad,*** *for being integral part in a life of journey and helped me out in all difficulties. I will always cherish the warmth shown by them.*

I express my deepest thanks to ***Srinivas, Senthil*** *and* ***Thangamani,*** *for selfless help and support during the research.*

I would like to extend my thanks to Technician ***Smt. Syamala Devi*** *and non teaching staff* ***Sirisha, Sowbhagyam*** *and* ***Kanaka Durga*** *for their help during the period of research.*

Heaven on earth is a choice we must make, not a place we must find. The days in ***Gannavaram*** *were like a dream without any worries. It would not have happened without the company of evryone.*

I extremely thankful to the Dean, ***Faculty of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati*** *and* ***Associate Dean, NTR College of Veterinary Science, Gannavaram,*** *for providing the facilities to carry out my research.*

Last but not the least, I would like to express my sincere gratitude to my beloved father ***Mr. China Venkateswara Rao****, loving mother* ***padma****, and caring sister* ***Mrs Chandra kala****. Guidance and moral support showered by them during*

hard phases of my life are the real blessings in my journey of life. Thanks for lifting me up when I was down, freeing me from cobwebs of worries and confusions.

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DECLARATION

*I **Dr. DANGETI V.V.N. DURGA PRASAD, I.D No: GVM/2014-051** hereby declare that the thesis entitled **"STUDY ON HISTOGENESIS OF KIDNEY IN SHEEP (Ovis aries)"**submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of MASTER OF VETERINARY SCIENCE is the result of original research work done by me. I also declare that the materials contained in this thesis have not been published earlier.*

Date

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ABSTRACT

The study was carried out in 45 embryos and foetuses of local Nellore sheep from 20 days to 150 days of gestation to explicate the development of various structures in the kidney. At 20 days (0.7cm CRL) of embryo, the mesonephros located lateral to the testis and occupied larger part of the coelomic cavity. In group I (39.5 days) to group III (150 days) the dorsal surface of both the metanephric kidneys was in contact with psoas major muscle. The metanphric kidneys, in all age groups were varying in colour from light to dark brown. In 20 day (0.7cm CRL) old sheep embryo

the pronephros was placed on either side of the dorsal aorta which was surrounded by undifferentiated mesenchymal cells. The pronephros consisted of only one giant glomerulus of 225±1.3µm in size. At 24 days (1.1cm CRL) degenerative changes were noticed in glomerular network due to which vacuolation was noticed between the tuft of glomerulus. In 20 day (0.7cm CRL) old embryos the mesonephric parenchyma was densely packed with tubules, migrating angioblasts and differentiating glomerulus. At 24 days (1.1cm CRL) the mesonephric glomerulus surrounding visceral and parietal layers were demarcated at this age. In 27 days (1.7cm CRL) the manifestation of macula densa of DCT, Juxtaglomerular cells of afferent arteriole and mesengial cells at vascular pole of Bowman's capsule was observed. At 49 days (7cm CRL) of gestation glomerulus shrinkage was noticed indicating the degeneration process initiated in mesonephros. The ureteric bud of metanephros was formed by evagination of the caudal part of the mesonephric duct in sheep embryo at 20 days of gestation (0.7cm CRL). At 44 days (5cm CRL) of gestation the number of the renal corpuscles were increased and distributed towards periphery from the pelvis. Demarcation between proximal and distal tubules was noticed. Major and minor calyces were apparent at 57 to 60 days of gestation and increased quantity of collagen at the sinus around the major and minor calyces. Loop of Henle was first appeared at 83.5 days, which were well established in juxta medullary nephrons as descending and ascending limbs. In group III the typical cortex and medulla was differentiated as adult kidney and renal corpuscles were still under differentiating stages in the cortical region by 101 days. Papillary ducts were lined by stratified cuboidal epithelium minor calyx was lined by transitional epithelium like adult kidney and smooth muscle were also noticed in the wall of the calyx minor in 115 days of gestation. By 120 days Juxta glomerular cells exhibited the cytoplasmic

granules. In group III at 101 days (26cm CRL) the capsule and tubules showed mild PAS positive reaction and the cytoplasm of collecting ducts showed intense PAS positive indicating the neutral mucopolysccharides in the collecting ducts. In group I to group III the renal corpuscles, tubules and collecting ducts showed negative reaction for both acid and alkaline phosphatases.

LIST OF SYMBOLS AND ABBREVATIONS

cm	:	Centimeter
CRL	:	Crown Rump Length
DCT	:	Distal Convoluted tubules
et al.	:	Et Alia
Fig	:	Figure
H&E	:	Haematoxylin and Eosin
mg	:	Milli Gram
mm	:	Milli Meter
PAS	:	Periodic Acid Schiff's Stain
PCT	:	Proximal convoluted tubules.
SPSS	:	Software Package used for Statistical Analysis
&	:	And

%	:	Per Cent
±	:	Plus or Minus
µm	:	Micro Meter

CHAPTER – I

1.0 INTRODUCTION

Sheep plays a significant role in the agricultural economy of several tropical countries and occupies unique position among livestock. Down through the centuries, rearing of sheep has remained a primary occupation for a vast section of the rural population in India. Sheep is a multi-facet utility animal for meat, wool, hides and manure which contribute greatly to the economy, especially in the arid, semi-arid and mountainous area, where crop and dairy farming are not economical.

According to 19th livestock census (2012), India's sheep population is 68.8 millions with a share 7.14 per cent of world sheep population. Besides its conventional usages, it has become an important research tool for new and advanced technologies, such as transgenic bioreactors, cloning the livestock and as a source of by-products.

Nellore sheep is the tallest breed among the Indian breeds and is widely distributed in Andhra Pradesh. It is a popular mutton breed in the southern regions of the country. Sheep rearing is a major source of income for landless labor and small

and marginal farmers of semi-arid regions in Andhra Pradesh (Ekambram *et al.,* 2013). Andhra Pradesh stands first in the sheep population with 26.1 million.

The urinary system in sheep is derived from the urogenital ridge of intermediate mesoderm. The pronephros, mesonephros and their tubules develop from the nephric ridge and the permanent kidney begins to develop when the ureteric bud extends into the metanephric mesenchyme.

Histomorphological studies on the development of various organs in sheep have been carried out extensively but very few studies have been conducted on developmental changes of the Nellore sheep foetal kidney in this area. However, there is lack of much information on the prenatal development of sheep kidney. The present work may be useful for the physiologists and clinicians to understand the functional ability during embryonal stages, to understand and interpret the congenital anomalies if any. Hence the present study was undertaken to bridge the gap in the existing information with the following objectives.

Objectives:

1) To study the gross morphology of kidneys at various stages of development in sheep foeti.

2) To study the histoarchitecture and histochemical changes in kidney during prenatal period.

CHAPTER II

2.0 REVIEW OF LITERATURE

2.1 Morphogenesis of kidney

2.1.1 Location

Malik and Vais (1998) noticed that the kidneys of goat were situated below the lumbar transverse process, left kidney was farther caudal in position and both kidneys were shifted rosrtally with advancement of age. The rostral shift of kidneys during development was due to relative growth variation of growing viscera.

The foetal kidneys in all cross bred pigs were located below the lumbar transverse process except foetuses of 86-114 days of gestation where the kidneys were detected more cranially (Sarma and Ahmed, 2007).

The kidneys of one humped camel were located below the transverse process of lumbar vertebra on each side, with the right kidney being more cranial in camel foetuses of three trimesters (Bello *et al.,* 2013 and Morovatisharifabad and Salehi ., 2015).

Jafar (2014) described that the metanephros appeared and was located relatively in the sacral region. It was closely associated with testes in 7 - 8 cm CRL (60 to 65 days) buffalo foetus.

2.1.2 Colour and shape

Malik and Vais (1998) revealed that the shape of kidneys was irregularly ovoid upto 10 cm CRL and changed to bean shape with rounded extremities in goat foetuses of 20 cm CRL onwards.

Bello *et al.* (2013) and Morovatisharifabad and Salehi (2015) observed that both the prenatal kidneys in camel were irregularly elongated and reddish brown in color when observed *in vivo*. In 90 days foetus, the elongated kidneys became bean shape. They were smooth and covered with a thin fibro-muscular capsule. From 60 to 120 days, the kidneys were attached to the posterior extremity of the adrenal glands on respective sides. Each kidney had both cranial and caudal surface, medial and lateral border and an upper and lower pole. The lateral border was convex in shape, medial border was concave and intended at the hilus. Adipose tissue surrounded the hilus and sides of the kidney.

Jafar (2014) described that the metanephros appeared and was located relatively in the sacral region, small in size, had smooth surface, covered by a thin fibro-muscular capsule, bean shaped and was considered to be immature organ in 7 - 8 cm CRL (60 to 65 days) buffalo foetus.

2.1.3 Morphometry

Gopinath *et al.* (1997) observed unnoticeable difference in respective measurements of right and left kidneys within the goat foetuses at all ages (0-150 days) but recorded a continuous increase in length, width thickness and weight with concomitant increase in the body weight and crown-rump length (CRL) of the foetus with advancement of age. Highly significant correlation was observed between the growth of the gland and growth of the foetus. The relationship between the length of the kidney and CRL of the foetus was almost straight.

The right kidney was heavier (11.30gm) than the left (10.66gm) in all age group foetuses. All the parameters of right and left kidneys varied in different age groups. The length, thickness and cross sectional area of right kidney were greater than those of left except width, which was more in left kidney up to 20 cm CRL goat foetuses (Malik and Vais, 1998).

Salehi and Morovathisharifabad (2012) observed the variation in growth between the left and right kidneys of camel in terms of length and width at 45 days and 60 days of gestation. Significant variation was recorded between the right and left kidney in terms of thickness at 45 days and 120 days of gestation. Maximum growth in length of 34.3% and 36% was observed at this age in right and left kidneys respectively, the growth being more in the left one. The width of right and left kidneys increased by 21.2% and 24.7% respectively. Similarly, the highest growth percentage of thickness was documented in right kidney (19.8%) and left kidney (21.6%) in 90 days foetuses, which clearly indicated greater embryogenesis of both kidneys in the early stages of development.

As per Bello *et al.* (2013), there was increase in the biometrical parameters of both the kidneys with advancement of gestational age in one humped camel from 1st to 3rd trimester. The weights of left and right kidneys were equal in first trimester fetuses and no significant difference was observed between them. The length and width of right kidneys were significantly greater than the left ones. However, all the parameters were greater in left kidneys than the right ones in second and third trimester.

2.2 Histogenesis of Kidney

2.2.1 Pronephros

Fraser (1920) studied the inter-relationship between pronephros and mesonephros in cat and concluded that the two organs developed in a strictly cranio-caudal sequence with no sharp dividing line between them.

Davies (1951) reported that the intermediate cell mass at the 7th segment and for some distance caudally, presented a well-marked bulge dorsally in sheep foetus. It was referred to as a 'nephric ridge' since the pronephric structures were doubtful. The nephric ridge deeply indented the overlying ectoderm. The differentiated nephric ridge and the intermediate cell mass outstripped that of the somites and extended for a considerable distance into the unsegmented part of the embryo. The intra-embryonic mesoderm was very bulky and was differentiated from the embryonic mesoderm by the development of a shallowdorsal groove by which it was demarcated from the unsegmented paraxial mass. At 11- 12 somite stage, the nephric ridge showed differentiation and separation from its dorsal margin of a rod of cells and the appearance of vesicles was initiated. They were in contact above with the primordium of the duct and merged ventrally with the intermediate cell mass. The duct primordium was not separated from the nephric ridge throughout its length but merged frequently with the dorsal wall of the vesicles or with the nephric ridge between them. The duct primordium was closely applied to the ectoderm at about the level of the future 14th somite. Pronephros persisted until late stage of embryonic life.

According to Gopinath and Singh (1998), there was aggregation of loose mesenchymal tissue at the cranial end of the mesonephros, around pre-existing pronephric ducts thus representing its site of occurrence in 6 mm CRL (15 day) goat embryos. The area of pronephros changed into an organized mass of fibrous tissue by 10 mm CRL (20 day) stage with the progressive condensation of mesenchymal cells.

2.2.1.1 Pronephric glomeruli

As per Davies (1951), at 14 somite stage in sheep embryo, the nephrogenic cord showed intense angioblastic activity and development of the glomerular complex. About fourteen vesicles were observed in the nephric ridge dorsal to nephrogenic cord. The vesicles were not metameric and possesed a basement membrane on all sides except on the ventral side. In 23 somite stage, the glomerular complex was located approximately from 7th to 14th or 15th somite, and was almost completely developed. The epithelium over the glomerular septa was still cuboidal. However, there were few connections between the glomerular capillaries and the dorsal aortae.

At 29 somites stage, the glomerular complex was almost fully developed and probably functional as judged by the histological and anatomical appearances. It was well supplied by endothelial outgrowths from the aorta or from its dorsal segmental and ventral intestinal branches. The cranial tubules already showed an increase in length and an early differentiation into a secretary and a collecting segment. The epithelium investing the glomerular capillaries in the cranial half of the glomerular complex was changed to a flat squamous layer.

2.2.1.2 Pronephric tubules

At 23 somites stage of sheep embryo, the associated tubules (about twenty-five) were short and entirely dwarfed by the massive glomerular pronephric complex that developed ventrally. There were about forty-five tubules in pronephros in various stages of development. The most rudimentary tubule was present at the caudal most end (Davies, 1951).

2.2.2 Mesonephros

The mesonephros in 6 mm CRL (15 day) goat foetus occupied a large part of coelomic cavity from below the 7th to 21st body somites and possessed well-defined

globular shaped mesonephric vesicles (26.6 μ). Many of these vesicles showed ventral elongation representing progenitors of mesonephric tubules. The cranial zone of mesonephros adjoining the area of pronephros contained short mesonephric tubules without any sign of their glomerular attachments. The middle zone (from below the 9th to 13th somites) showed dorso-ventrally oriented columns of angioblasts, separated from each other by narrow gaps (Gopinath and Singh, 1998).

Suman *et al*. (2013) described the mesonephros in buffalo foeti from 2.5 cm CRL onwards. It extended on the dorsal part of the coelomic cavity in the close vicinity of gonads. Mesonephric vesicles were observed in caudal mesonephros from 3.0 cm CRL onwards. The lining epithelium was simple squamous at 3.0 cm CRL and changed to cuboidal at 3.5 cm CRL. The shape of the vesicles was either round or oval. The cranial part of mesonephros was narrow while the caudal end was broad and showed mesonephric vesicles, tubules and glomeruli of different shapes.

2.2.2.1 Mesonephric glomeruli

As per Gopinath and Singh (1998), the goat embryos of 14 mm CRL (24 day) showed the whole of the cup of Bowman's capsule nearly filled with glomeruli in the mesonephric corpuscle resembling that of adult kidney. Blood capillaries of different diameters were noticed in the intertubular tissue at this stage. The glomerular masses filled the cavity of Bowman's capsule completely by 18 mm CRL (30 days). The proximal convoluted tubules were funnel shaped to accommodate the capillary tuft and served as the primordium of mesonephric glomeruli. Simple columnar epithelium lined the glomeruli forming the parietal layer and simple squamous epithelium lined capillary tuft as visceral layer, separated from each other by large Bowman’s space. Glomeruli had two types of cells namely angioblastic and mesenchymal cells. Angioblastic cells were having darkly stained rounded nuclei in the vacuolated

cytoplasm and the mesenchymal cells were spindle shaped with large nuclei. The number and size of mesonephric glomeruli increased with increase in the size of foetus from 2.5 cm to 4.1 cm CRL.

2.2.2.2 Mesonephric tubules

Most of the mesonephric tubules in goat embryos showed histological differentiation into the proximal and distal segments. The former were lined with simple columnar epithelium (9.9 µ height) and studded with brush border at luminal surface, while the latter presented simple cuboidal cells without any evidence of brush border (Gopinth and Singh, 1998).

Suman *et al.* (2013) stated that the mesonephric tubules of buffalo foetus occupied the greater area of caudal part of mesonephros at 3.0 cm CRL and were differentiated into proximal and distal convoluted tubules. The proximal convoluted tubules were oval in shape and were lined by low columnar type of epithelium along with eosinophilic striations whereas distal convoluted tubules were rounded and were lined by simple cuboidal type of epithelium. The length and width of proximal convoluted tubules varied from 255 to 355 µ and 130 to 185 µ in 2.5 cm and 4.1 cm CRL foeti respectively.

2.2.2.3 Degeneration of mesonephros

Chandra (1964) and Gopinath and Singh (1998) observed complete degeneration of mesonephros in goat foetus at 84 and 95 mm CRL, respectively.

Gopinath and Singh (1998) also stated that the structural distortion of mesonephric tubules marked the onset of degenerative process in cranial zone of the organ in 18mm CRL (30 days). The degenerative changes advanced in cranio-caudal order to middle and adjoining one-third part of the caudal zone of mesonephros of 22 mm CRL (34 day) goat foetus.

The cranial part of mesonephros degenerated earlier than the caudal part in buffalo foeti of 3.5 cm CRL. Some of the mesonephric glomeruli lost their walls and large masses of tufts of glomeruli were observed in the cranial mesonephros of 5.7 cm CRL. The degenerative changes were more marked in buffalo foeti of 7.2 cm and 10.3 cm CRL and advanced stages of degeneration in vesicles, tubules and glomeruli of the mesonephros were observed at 10.3 cm CRL (Suman *et al.,* 2013).

2.2.3 Metanephros

Tiedemann (1976) reported the onset of metanephric kidney development in 27 to 30 days sheep embryos. The origination of metanephros started in 21 to 28 days pig embryo (Horster *et al.,* 1999); 139 to 166 days elephant embryo (Gaeth et al., 1999); 25 days cow embryo (Hyttel *et al.,* 2010) and 47 days buffalo embryo (Suman *et al.,* 2008). Patten (1949) in pigs and Arey (1962) in man described the origin of metanephros from caudal part of mesonephric duct in the embryos of 5mm CRL.

According to Gopinath and Singh (1999), the internal zonation of metanephros into the medulla and cortex became obvious with peripheral shift of the blastema in goat embryos of 24 mm CRL (36 days). The aggregates of blastema cells below ampullated end of cortical ducts elongated into S- shaped vesicles and consisted of a lower and an upper tubular limb in embryos of 35 mm CRL.

As per Sarma and Ahmed (2007), no renal pelvis was evident in metanephric kidneys of 41-55 days pig embryos. The renal pelvis was distinct and extensive with well developed ureter that originated from the middle of the pelvis from 56-70 days of gestation onwards. In the foeti of 75-81 days, the cortex was first observed and gradually became larger than the medulla. The pelvis was well demarcated from the medulla with a sharp demarcating line in 101 -114 days foetuses.

Suman *et al*. (2008) noticed that the metanephros developed dorsal to the caudal part of mesonephros in the buffalo foeti of 3 cm CRL. The differentiation of cortex and medulla occurred in the metanephros at 5.7 cm CRL. The cortex was larger in size and had metanephric glomeruli, proximal and distal convoluted tubules.

Jafar (2014) described that metanephros was histologically not yet differentiated into cortex and medulla in 7-8 cm CRL (60-65 days) buffalo foetus. The metanephros at this stage revealed many vesicles at different stages of development like comma-shaped bodies, S-shaped bodies and immature glomeruli with relatively few collagen fibers. The metanephric parenchyma was poorly developed and majority of it was found covering the external surface.

2.2.3.1 Ureteric bud

According to Gopinath and Singh, (1998), the duct system of metanephros in goat developed from the ureteric bud. The latter evaginated from the caudal part of the mesonephric duct in embryos of 8 mm CRL (17 days) and grew cranial over the mesonephros by 12 mm CRL (21 days). It's distal end was ampullated and capped by nephrogenic blastema at this stage of development. The epithelial cells lining the whole length of this primordial duct stained lightly with eosin. Their nuclei were highly basophilic and stacked in 2-3 layers. The nucleoli occupied a central position in light stained area of chromatin material. With little change in histomorphology, the ureteric bud in goat embryos of 14 mm CRL (24 days) elongated cranio caudally at ampullated end to differentiate into 2 primary branches by 18 mm CRL (30 days) as forerunners of intra renal duct system. The epithelium lining these ducts resembled that of the earlier stages in cellular and nuclear details. The terminal end of each branch was surrounded by nephrogenic blastema.

2.2.3.2 Metanephric blastima

Chandra (1964) observed the metanephric blastema around ampullary end of ureteric bud in goat embryos of 13 - 19 mm CRL. Gopinath and Singh (1999) reported that the nephrogenic blastema differentiated from mesenchymal cells and surfaced over the ampullated end of the ureteric bud in goat embryos of 12 mm CRL (21 days). Their nuclei stained deeply compared to surrounding mesenchymal cells. The relative density of blastema cells was more towards caudal than cranial end of the ureteric ampulla. At certain places, the undifferentiated mesenchymal cells invaded from the periphery deep into the metanephric mass of blastema in embryos of 14 mm CRL (24 days). Concurrent to the bifurcation of the ureteric bud at terminal end into branches of primary order, the blastema increased in amount and grouped around terminal end of each duct at 18 mm CRL (30 days). The mesenchymal cells bordering whole of the nephrogenic mass in these embryos condensed at periphery as primordial capsule of the developing gland.

2.2.3.3 Renal Corpuscle

Gopinath and Singh (1999) described that the nephrons of goat developed first in the juxta-medullary zone at 45 mm CRL (50 days) and subsequently in the intermediate and sub capsular zones of the cortex in embryos of 55 mm CRL (52 days) and 70 mm CRL (56 days) respectively. The Bowman's capsule, was spheroidal in shape and housed vascular glomeruli in embryos of 120 mm CRL (74 days). Their visceral and parietal surfaces were lined with simple cuboidal epithelium. The glomeruli were of larger size in juxta-medullary (151.8±4.9 micron) than in the intermediate (116.4±1.6 micron) and subcapsular (91.6±4.6 micron) zones. Macula densa and group of mesangial cells at vascular pole of a Bowman's capsule indicated that the metanephros attained nearly an adult kidney like structure in this age group. The epithelial cells lining the visceral and parietal walls of the Bowman's capsules

reorganized into simple cuboidal and simple squamous epithelium in 230 mm CRL (107 days) foetus. The mesangial cells were grouped at vascular pole of the renal corpuscles between glomerular arterioles and macula densa.

Suman *et al*. (2008) demonstrated the metanephric glomeruli in buffalo foeti along with the metanephric tubules at 4.1 cm CRL. The glomeruli were enveloped by parietal and visceral epithelial layers of Bowman's capsule. They also noticed that the distal convoluted tubules lied nearer to the visceral pole of metanephric glomeruli which may be transformed into the future macula densa. The glomeruli were mostly crescentic in shape and the renal corpuscle showed no Bowman's space at 10.3 cm CRL, the juxtamedullary glomeruli identified together with macula densa, juxtaglomerular cells of afferent arteriole and mesangial cells were accounted at 32.5 cm CRL and glomeruli became rounded with a definite Bowman's space in the renal corpuscle at 44 cm CRL onwards.

According to Jafar (2014), the beginning of nephron formation occurred during 60-65 days of gestation in bovine. The metanephric mesenchyme responded to the invading bud by proliferation and condensation to form coronas of cells around each branch of the bud. The cells within the condensed mesenchyme were transformed into epithelial cells. The epithelia formed oval shaped structures called renal vesicles and this was considered as the first step in nephron development. These vesicles were gradually elongated and convoluted to form an elongated tubule which was later converted into the comma-shaped body representing the second stage of nephrogenesis. As development progressed, the comma-shaped body transformed into next stage of nephron development called S-shaped tubule stage. The lower limb of S-shape tubule was situated furthermost from the branch of collecting tubule just below the lower cleft with vascular mesenchyma contained within the concavity of the lower

S-curve. It had long been recognized as glomerulus. The double walled hemispherical structure which developed from the greater part of the lower S-curve formed the inner and the outer layers of Bowman's capsule. The space between the two layers that continued with the lumen of the tubules formed Bowman's space. Finally, the maturing glomerulus stage was oval or rounded.

Bello *et al.* (2013) described that the cortex of kidney in camel below 130 days of gestation was found to have numerous mesenchymal cells and connective tissues with few developing glomerulus in the first trimester. The number of mesenchyme cells was reduced in the second trimester (131-260 days). Few fully developed glomeruli and immature developing duct system were observed at this stage. Camel is the only domestic animal that had collecting duct system in the cortex. In the third trimester (260-390 days), there were numerous developed glomeruli, mature duct system and connective tissue.

2.2.3.4 Metanephric tubules

Gopinath and Singh (1999) studied that the proximal tubules arose in continuation with the Bowman's capsule from lower limbs. The nephrons developed first in the juxta-medullary zone at 45 mm CRL (50 days). The distal convoluted tubules had their origin from the upper limbs of the primordial vesicles. Considerable coiling and epithelial specialization was observed in 70 mm CRL (56 days) foeti. The Henle's loop and distal convoluted tubule originated from the middle and upper limbs of the primordial vesicles at this age. The proximal convoluted tubules and distal convoluted tubules appeared better developed and more coiled in goat embryos of 120 mm CRL (74 days) and the epithelial cells lining them stained deeply with eosin. The proximal convoluted tubules of goat foeti formed bulk of the cortex at 250 mm CRL (112 days) and segments in Henle's loop were formed at 118 days of gestation.

As per Suman *et al*. (2008), the metanephric tubules developed from the mesenchymal cells of the metanephrogenic mesoderm at 3.0 cm CRL of buffalo foeti. The peripheral parts of these tubules were dilated and showed dichotomous bifurcation of the tubules. The branches of all the tubules were lined by multilayered epithelium with elongated nuclei. There was no clear differentiation between renal cortex and medulla at this stage, but the formation of S-shaped vesicles along with metanephric tubules could be seen. The PCT and DCT were first observed in 4.1 cm CRL buffalo foeti and were first formed in the juxtamedullary area. Proximal convoluted tubules (PCT) were lined by simple columnar epithelium upto 5.7 cm CRL stage. The tubular segments of nephron were clearly differentiated in the cortex as proximal and distal convoluted tubules in the buffalo foeti of 5.7 cm CRL onwards. PCT appeared in the intermediate and sub capsular areas of the metanephros in 10.3 cm CRL onwards. Formation of striations (future brush border) at the luminal end of the epithelial cells was first noticed at the age of 11.2 cm CRL and the juxtamedullary glomeruli were encapsulated. In 23 cm CRL foeti, PCT were lined by simple cuboidal to pyramidal type of cells along with luminal striations. The DCT were lined by simple cuboidal epithelium with relatively less eosinophilic cytoplasm and darkly stained nuclei. The DCT presented nuclear clustering, which was regarded as the future macula densa of the juxtaglomerular apparatus.

Jafar (2014) observed that as development progressed, the upper and middle part of the S-shaped tubule remained tubular and elongated, generated the proximal tubule, loop of Henle's and distal tubule that joined the ureteric branch to develop into the collecting ducts. Henle' loop was noticed at the foetal age of 7-8cm CRL (60 to 65 days) in buffalo. At 90-95 days of gestation, the proximal and distal convoluted tubules of buffalo foeti were differentiated completely and were lined by irregular

cuboidal epithelium. The epithelial cells were light in colour with rounded, oval or irregular large nuclei and girdled by thin basement membrane. The cells had no brush border. The lumen of the distal tubule was comparatively narrow. Henle's loop was undifferentiated relatively at this age. In 120 to 130 days buffalo foeti, the proximal tubule was lined with simple cuboidal epithelium and the cells had rounded large nucleus, were light in colour and composed of thin and thick part with no brush border. The distal tubule cells were changed to tall cuboidal cells with rounded dark nucleus. The Henle's loop become visible and consisted of two parts. The first thin segment was lined by squamous cells which bulge into the lumen while the second thick segment was similar to proximal convoluted tubule and was lined by low cuboidal cells.

Jafar (2014) studied 60 to 65 days buffalo foetus and observed that the ureter was elongated and split into cranial and caudal evident portions (primary branches), which represented the future major calyces. The beginning of duct system induction was observed with up to ten funnel-shape divisions referred to as minor calyces. At 90 to 95 days of gestation, the para-sagittal section of metanephros appeared with visible medullary rays and was composed of collecting tubule and collecting duct (duct of Bellini) that terminated with papilla. The collecting duct in the cortex was lined with simple cuboidal epithelium and was gradually translated to the columnar epithelium in medulla until it became pseudostratified epithelium at the papillary duct. At 100 to130 days of gestation, more evidence of collecting tubules and collecting duct was observed. There was increase in the diameter of individual collecting tubules and length of the loops of Henle, individual collecting tubules and collecting ducts in 150-160 days foeti.

2.2.3.5 Metanephric Capsule

Bello *et al.* (2013) described that the kidneys had smooth surface and was covered by a thin fibro-muscular capsule in camel of all age groups that tended to become withered with the advancement of gestational age. Whereas, Jafar (2014) in 7 - 8 cm CRL (60 to 65 days) buffalo foetus and Morovatisharifabad and Salehi. (2015) in camel at 90 days of gestation reported the same observations.

Tanvi *et al.* (2015) found that the mesenchymal cells of goat foeti condensed as a thin layer around the kidney (metanephros) as forerunners of the renal capsule at 45 days (CRL 5.3 cm) and 46 days (CRL 6.3 cm) of gestation. Gradually, the mesenchymal cells differentiated into the fibroblasts and secreted few reticular fibres mainly towards the periphery of the capsule. The relative amount of the reticular fibres increased at 48 days (CRL 7.5 cm) and 50 days (CRL 7.6 cm) of gestation. At 58 days of gestation the stromal elements were composed mainly of mesenchymal cells and fibroblasts in goat foeti. Nerve fibres were also observed in the stroma. Collagen fibres were observed in the capsule at 61 days (CRL 8.8 cm) and 62 days (CRL 10.1 cm) of gestation and were arranged in thick bundles at 96 days of gestation (CRL 19.0 cm). Nerve fibre strands were noticed mainly towards inner parts of the capsule. At 96 days of gestation, the stromal elements were composed mainly of densely arranged mesenchymal cells especially in the medulla. At 109 days of gestation (CRL 25.0 cm), capsule contained differentiating mesenchymal cells, fine elastic, reticular and thick collagen fibres. Blood vessels of various sizes were observed. Large aggregations of nerve fibres could be noticed in the stroma (Tanvi *et al.* 2015).

2.3 Histochemistry

Gopinath and Singh (1999) reported that the epithelial cells of larger ducts, both in the cortical and medullary areas, showed uniform sudanophilia in the

infranuclear part from 180 mm CRL onwards (96 days). Epithelial cells of proximal and distal convoluted tubules in 290 mm CRL (130 days) goat embryos and beyond showed uniform sudanophilia. The ducts of all orders showed gradual increase in staining intensity of sudanophilic fat in 290 mm CRL (130 days) and beyond.

2.3.1 Neutral Mucopolysaccharides

According to Gopinath and Singh (1998), at 33 mm CRL (45 day) in goat embryos, most of the tubules and glomeruli exhibited reduced affinity for eosin and PAS stains. The epithelial cells lining the tubules showed faint PAS-positive activity at luminal surface. In 160 mm CRL (89 days), many of the ducts were lined with simple columnar epithelium, the nuclei being placed apically in them. The epithelial cytoplasm in ducts of all orders possessed PAS - positive granules with a little more aggregation in the infranuclear part of ducts of medullary area. The epithelial cells of larger ducts, both in the cortical and medullary areas, showed heavy concentration of PAS-positive material in the infranuclear part from 180 mm CRL onwards (96 days). At 107 days, the cytoplasm in cells of macula densa showed mild to moderate PAS-positive granules. The epithelial cells lining the proximal convoluted tubules of goat foeti showed PAS-positive brush border at free surface by 250 mm CRL (112 days). The epithelial cells of proximal and distal convoluted tubules in 290 mm CRL (130 days) goat embryos and beyond showed a little more concentration of PAS-positive material in supranuclear part. The ducts of all orders showed gradual increase in staining intensity of PAS- positive material in 290 mm CRL (130 days) and beyond. The large PAS- positive granules were noticed in infranuclear zone of epithelial cells lining the collecting ducts.

As per Suman and Bansal (2007), a weak reaction of neutral mucopolysaccharides was observed in the capsule and glomeruli, whereas the

proximal convoluted tubules (PCT) showed a moderate reaction in the buffalo foeti at 11.2 cm CRL. Distal convoluted tubules (DCT) and loop of Henle did not reveal any reaction. The excretory ducts in the medulla also showed a weak to moderate PAS positive reaction. At 21.5 cm CRL capsule, glomeruli and PCT showed moderate to strong PAS- positive reaction whereas the distal convoluted tubules and loop of Henle showed weak reaction. A moderate to strong PAS reaction was observed in the capsule and glomeruli in 44-89 cm foeti whereas weak reaction was observed in distal convoluted tubules and loop of Henle. A strong PAS- positive reaction was observed in the basement membrane and striations (brush border) of PCT at 89 cm CRL buffalo foeti.

2.3.2 Alkaline Phosphatase

The luminal areas of cells in the mesonephric proximal tubules and metanephros in early gestation stained deeply for alkaline phosphatase in sheep (Davies, 1952).

Chapter III

3.0 MATERIALS AND METHODS

The present study was conducted on 45 local Nellore sheep embryos and foetuses. The embryos and foetuses were collected immediately after slaughter from the abattoirs located in and around Vijayawada, Andhra Pradesh. The foetuses were randomly collected from 20 days (0.7 cm CRL) to 150 days (42 cm CRL). Among these 45 foetuses, 31 were male, 7 were female and 7 were undifferentiated grossly.

The approximate age of embryos and foetuses was determined on the basis of their CRL upto 3 cm CRL size (Bryden *et al.,* 1972) (Table 3.1) and for later ages by using the following formula (Hejazi *et al.,* 2011).

X= 2.74Y+30.15

Where 'X' was the age of the foetus and 'Y' was the crown rump length of the foetus in cm. The specimens collected were divided into the following three groups based on their age (Table 3.2).

Table 3.1: Showing various age groups of embryos/ foetuses based on their crown rump length (CRL)

Sl No	CRL (cm)	Gestational age (days)
1	0.7	20
2	1.1	24

3	1.5	25
4	1.7	27
5	1.9	29
6	2.0	31
7	3.0	35.5

Table 3.2: Showing various age groups of embryos/foetuses based on their crown rump length (CRL)

S. No	Age groups of embryos	Number of embryos / foetuses used
1	Group I (0 to 50 days)	20
2	Group II (51 days to 100 days)	18
3	Group III (101 days to full term)	7

The crown rump length (CRL) was measured from the most anterior part of the crown to the base of the tail.

3.1 Morphology and morphometry

After measuring the crown rump length, the foetuses were dissected, the gross anatomical positions of kidneys were noted and proceeded for recording the morphological details (weight, length and width) by using monopan balance and Vernier callipers. Since the kidneys were appeared very as small in group I (until 30 days) unable to measure. Mesonephros and metanephros were measured in group I (from 39 days), II and III. Morphometric analysis was carried out by paired t-test, using SPSS. Graphical representation was done by using Microsoft Excel.

3.2 Histological study

The kidneys could not be separated from the body for recording morphometric details from 20 days (0.7 cm CRL) to 35 days of gestation (3 cm CRL). Serial sections were made for histogenesis.

The embryos of 39 days onwards, after recording the morphological details, kidneys were fixed in 10% Neutral buffered formalin and Bouin's fluid (Luna, 1968). The fixed tissues were processed and paraffin sections of 3-5 µm thickness were stained by the following methods for histological study.

1. Haematoxylin and Eosin (H&E) stains for routine histological study (Singh and Sulochana, 1996).
2. Masson's Trichrome staining method (Luna, 1968).
3. Verhoef's and Weighert's techniques for elastic fibres (Singh and Sulochana, 1996).
4. Wilder's method for reticulum Luna, 1968).
5. Bielschowsky's and Luxol fast blue methods for nerve fibers (Luna, 1968).
6. Jones silver stain for basement membranes of capillaries, tubules and larger vessels (Luna, 1968).
7. Harada's stain for Juxta glomerular granules (Harada, 1970).

3.3 Histochemical study

Frozen sections of 10-15 µm and paraffin sections of 3-5 µm thickness were subjected for the following histochemical studies.

1. Acid and alkaline phosphatases for enzymes (Luna, 1968).
2. Periodic Acid Schiff (PAS) technique for neutral polysaccharides (Brancroft and Gamble, 2003).

3.4 Micrometry

Thickness of pronephros, pronephric glomeruli, mesonephros, mesonephric glomeruli, mesonephric tubules, metanephros, metanephric glomeruli and metanephric tubules were measured by ocular and stage micrometer under light microscope. The statistical analysis was done by using SPSS software.

Chapter IV

4.0 RESULTS

The study was carried out in 45 embryos and foetuses of local Nellore sheep from 20 days to 150 days of gestation to explicate the development of various structures in the kidney. When open the foetuses, kidneys were located at different areas of abdomen and pelvic cavity in various gestation periods.

4.1 Morphogenesis of kidney

4.1.1 Location

In group I at 20 days (0.7 cm CRL) of embryo, the mesonephros located lateral to the testis and occupied larger part of the coelomic cavity, cranially continued by pronephros and caudally opened with the mesonephric duct into the urogenital sinus. Mesonephros was located between metanephric kidney cranio-medially and testes caudo-laterally by 39 days (3.2 cm CRL) (Fig.1). At 41 days of gestation (4 cm CRL), mesonephros was located at the base of the metanephric kidney, smaller than the metanephric kidney. Dorsal surface presented a mesonephric duct, traversing entire length of mesonephros and opened caudally into the urogenital sinus on both sides (Fig.2).

The mesonephros was further extended towards caudal in 42.5 days (4.5 cm CRL) and placed more caudal in 48 days of gestation (6.5 cm CRL) and medially in the middle of mesonephros related to testes in 50 days (Fig.3) which was slowly modified into ductus differens at 58 days (10 cm CRL) in male and in 60 days (12 cm CRL) uterine tubes in female (Fig. 4). Due to decrease in size by 41 days, the mesonephros and testes were pushed towards posterior end of metanephric kidney at 50 days in male foetus (10 cm CRL) and 60 days in female foetus (12 cm CRL).

Group I (39.5 days) to group III (150 days) the dorsal surface of both the metanephric kidneys was in contact with psoas major muscle. Metanephric kidneys were located cranio-medial to mesonephros and smaller than the mesonephros in 39.5 days (3.5 cm CRL) of embryo. Left kidney remained in contact with the primitive stomach on the cranio-lateral aspect. Half of the ventral surface of right kidney was in contact with very long caudate process of the liver. Postero-lateral surface was attached to the testes on either side. The metanephros were attached anteriorly to the posterior extremity of the adrenal glands at respective sides and each kidney showed both dorsal and ventral surfaces, medial and lateral borders and cranial and caudal poles. Cranial end was slightly larger than the caudal pole and the size of kidneys was increased in 41 days of gestation than earlier ages and the mesonephric ducts from each side approach the urogenital sinus, the two mesonephric folds fused between the bladder ventrally (Fig. 2). In the male 50 days (10 cm CRL) embryo the peritoneal fossa between the bladder and the genital cord becomes obliterated, but it persists in female as the utero-vesical pouch in 60 days (12 cm CRL). The mesonephric duct itself becomes the canal of the epididymis, vas deferens and ejaculatory duct.

4.1.2 Colour and Shape

At 39 days (3.2 cm CRL) the mesonephros were cream in colour and appeared as elongated oval shaped and its cranial border was rounded and caudal border was tapered (Fig.1) but it was elongated bulged cord like structure in 41 days (4cm CRL) (Fig. 2).

The metanephric kidneys, in all age groups were varying in colour from light to dark brown. The shape of metanephric kidneys were irregularly ovoid upto 39 days (3.2 cm CRL), smooth and covered with a thin fibro-muscular capsule, and changed to bean shape with rounded extremities at 41 days of gestation (4 cm CRL). Due to increase the concavity on the medial surface and appearance of medial fissure on the right kidney which appeared as triangular in shape. Left kidney was slightly curved in shape with broader anterior extremity than posterior extremity in 50 days sheep embryo (7.5 cm CRL) (Fig. 3). The renal hilus was extended on the ventro-medial aspect of the metanephric kidney upto 60 days (12 cm CRL) of gestation (Fig. 4). Later it was restricted to the medial surface and the ureters were traversing downwards to the bladder on both the kidneys until birth (Fig.4&5).

In 60 days (12 cm CRL) foetuses the right kidney was bean shaped with broader posterior extremity (Fig.4). The left kidney appeared as slightly curved bean. Both the kidneys were pale cream in colour. In the medial border was compressed to concave. The lateral border was thick at cranial part and comparatively thin at caudal part. The cranial extremity was wide whereas the caudal extremity was thin and flat 78days (17.5cm CRL) (Fig. 5). It was also surrounded by thick layer of peri renal fat but the colour of the kidney was drastically changed to brown in 145 days (Fig. 6).

4.1.3 Morphometry

In 39.5 days (3.2 cm CRL) of gestation, the mesonephros was appeared to be 0.8 cm in length greater than the metanephric kidney 0.4 cm (Fig.1). The size of mesonephros was reduced from 41 days onwards (Table 4.1).

Table 4.1 Length, width and weight of mesonephros in various gestational ages.

Sl. No	Age of the foetus (days)	Mesonephros		
		Average Length (cm)	Average Width (cm)	Average Weight (gm)
1	39.5	0.80	0.35	0.047
2	41	0.75	0.31	0.040
3	42.5	0.71	0.29	0.030
4	45.5	0.60	0.25	0.025
5	49	0.44	0.20	0.023
6	50	0.35	0.17	0.021
7	52	0.27	0.15	0.019
8	54.5	0.21	0.13	0.016
9	58	0.20	0.11	0.013

The metanephric kidney was gradually increased from group I to group III foetuses in all morphometric parameters (Table 4.2)

Table 4.2 Morphometic details of metanephric kidney in various age groups.

Sl. no	CRL (cm)	Age (cm)	Left kidney			Right kidney		
			Weight (gm)	Length (cm)	Width (cm)	Weight (gm)	Length (cm)	Width (cm)
1	3.2	38.9	0.53	0.27	0.12	0.53	0.25	0.12
2	3.5	39.5	0.56	0.27	0.12	0.55	0.26	0.13
3	3.7	40	0.58	0.29	0.14	0.57	0.27	0.14
4	4.0	41	0.62	0.31	0.14	0.62	0.29	0.14
5	4.5	42.5	0.68	0.36	0.15	0.65	0.33	0.15
6	5.0	44	0.71	0.41	0.18	0.69	0.40	0.18
7	6.0	46.5	0.76	0.49	0.22	0.73	0.45	0.22
8	7.0	49	0.81	0.54	0.27	0.79	0.53	0.26
9	7.5	50	0.86	0.60	0.30	0.83	0.60	0.30
10	8.0	52	0.86	0.61	0.32	0.83	0.60	030
11	9.0	54.5	0.90	0.66	0.34	0.89	0.64	0.33
12	10.0	57	0.93	0.76	0.46	0.91	0.75	0.45
13	11.0	59	0.95	0.82	0.58	0.93	0.81	0.57
14	12.0	60	0.97	0.93	0.60	0.95	0.92	0.59
15	15.0	71	1.01	1.35	0.80	1.00	1.30	0.79
16	16.0	74	1.10	1.50	0.85	1.09	1.49	0.83
17	17.5	78	1.15	1.61	0.89	1.13	1.58	0.85
18	19.5	83.5	1.19	1.76	0.99	1.17	1.72	0.98

19	21.0	87	1.24	2.00	1.10	1.23	1.95	1.09
20	24.0	96	1.69	2.20	1.50	1.65	2.2	1.60

Groups	Particular	Standard mean		Std.Deviation ± Std.Error	
		Left	Right		
I	Weight	0.6789	0.6622	0.11570±0.03857	0.10628±0.03543
	Length	1.1450	1.1126	0.29395±0.8486	0.29246±0.08443
	Width	4.4530	4.3971	2.65674±1.01549	2.63367±0.99543
II	Weight	0.3933	0.3756	0.12400±0.4133	0.12749±0.04250
	Length	1.3808	1.3600	0.62241±0.17967	0.61912±0.17872
	Width	3.7357	3.7071	1.33501±0.50459	1.30936±0.49489
III	Weight	0.1822	0.1822	0.6648±0.2216	0.06379±0.02126
	Length	0.8300	0.8258	0.40394±0.11661	0.42376±0.12233
	Width	2.6600	2.6886	1.01280±0.38280	1.07637±0.40683

21	25.5	100	1.75	2.37	1.53	1.74	2.36	1.53
22	26.0	101	2.14	2.5	1.56	2.10	2.48	1.51
23	27.0	105	2.40	2.7	1.74	2.40	2.7	1.73
24	28.0	107	2.85	2.90	1.99	2.83	2.88	1.97
25	31	115	3.29	3.10	2.20	3.25	3.10	2.19
26	33	120	4.10	3.95	3.52	4.10	3.89	3.90
27	42	145	7.3	4.9	3.61	7.1	4.9	3.52
28	44	150	9.10	6.10	4.00	9.00	6.00	4.00

Table: 4.2.1 Morphometric analysis of metanephric kidney.

Morphometric analysis of kidney revealed that there was no significant difference within the groups between left and right kidney and no significant difference in group I and group II in terms of weight and width, but there was significant difference of weight and width of both kidneys in group III. Significant difference was noticed in length in all the age groups (Table 4.2 & 4.2.1).

4.2 Histogenesis of kidney

4.2.1 Pronephros

In 20 day (0.7 cm CRL) old sheep embryo, at the level of body somite, ventral to the intermediate cell mass towards the dorsal median line, the pronephros was placed on either side of the dorsal aorta which was surrounded by undifferentiated mesenchymal cells (Fig.7). Pronephric vesicles were surrounded by a layer of mesenchymal cells and fibers (Fig.8). At 24 days (1.1cm CRL) of gestation the pronephros was surrounded by a thin layer of connective tissue capsule which was lined by squamous to cuboidal cells but the posterior pole of pronephros was lined by stratified cuboidal cells. On dorso-posterorily a large capillary was lined by simple squamous cells represented posterior cardinal vein (Fig.9). The degeneration of pronephros was first observed at cranial pole in 27 days (1.7 cm CRL) the mesenchymal covering around the pronephros was disappeared by leaving few tubules and rest of the pronephros was filled with differentiating mesenchymal cells and completed the process of degeneration by 31 days (2cm CRL) of gestation in sheep embryos.

4.2.1.1 Pronephric glomeruli

On either side of the dorsal aorta showed intense angioblastic activity in 20 day old embryos (0.7cm CRL). The pronephros consisted of only one giant glomerulus of 225±1.3 μm in size was placed medially and varied from middle to ventral in its location. The glomerulus was consisted of network of fine tubule like structures which were densely oriented and were surrounded by thin layer of cuboidal cells with basophilic nucleus and connective tissue fibres (Fig.8). These fibres were also found within the glomerulus. Discontinued irregular capsular space was noticed in and around glomerular corpuscle and was lined by squamous to cuboidal cells.

At 24 days of gestation (1.1cm CRL) the pronephric glomerulus was appeared at the cranial pole and occupied 1/3rd of total mass of pronephros. The size of renal

corpuscle was appeared to be reduced than the earlier age. The capillary density was decreased with increased size of endothelial cells (Fig.9). In giant glomerulus surrounded by a capsular space which was distinct at some places only. The pronephric glomerulus was surrounded by a single layer of squamous to cuboidal cells. At posterior pole these cells appeared as tall cuboidal cell indicated the tubular continuation. Degenerative changes were noticed in glomerular network due to which vacuolation was noticed between the tuft of glomerulus at this age. These degenerative changes were more advanced in the glomerulus, with irregularly interspersed of glomerular network and the glomerulus was surrounded by blood islands in 25 days (1.5cm CRL) (Fig.10). Glomerulus do not develop in association with the cranially situated nephric tubules which ultimately disappeared and complete degeneration of glomerulus was noticed by indistinct glomerulus in the pronephros at about 27 days (1.7cm CRL) (Fig.12).

4.2.1.2 Pronephric tubules

At 20 days sheep embryo (0.7 cm CRL) many tubules were present in the pronephros, some were fully formed and some were incomplete and open type. Periphery of the pronephros consisted of irregular sized tubules, varied in size. The tubules present at dorsal end of the most periphery of the vesicles were elongated and irregular in shape, which traversing towards the caudal part of the vesicle. These tubules were lined by cuboidal cells with round, centrally placed nucleus and were surrounded by a thin layer of connective tissue cells and fibers. Tubule like structures present close to the glomerulus were appeared as incompletely oriented cluster of angioblast cells. The caudal ends were connected with coelomic epithelium by cellular strands like stalk which probably present the rudimentary peritoneal funnels

on either side. In between and surrounding the tubules undifferentiated mesenchymal cells and fibers were present (Fig.8).

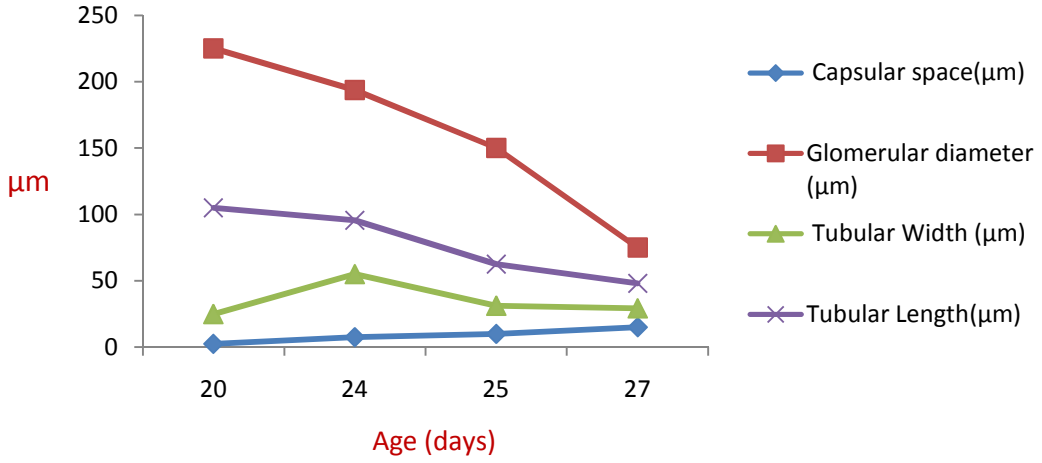
At 24 days (1.1cm CRL) the mesenchymal differentiation was progressed than the earlier age, the number of tubule were 6 to 7 restricted to caudal end of pronephros and these tubules were lined by cuboidal cells with discontinuous brush borders. The lumen of the tubules was occupied by eosinophilic material. The sizes of the tubules were increased upto this foetal age and were located postero-dorsally to the glomerulus. The cellular strands elongated little beyond into the coelomic cavity (Fig.9) (Table.4.3). Some tubules present with brush borders and little narrow lumen when compared to the other tubules without brush border (Fig.10).

Increased concentration of cellular strands at the caudal end and further increased the length of the stalk indicated the union of pronephros with primary excretory duct, which appeared as solid rod of cells in the coelomic cavity. Tubules showed eosinophilic material in it and lack of nerve fibres (Fig.11). The size of the tubules was started decreasing at 25 days than the earlier gestation periods (Table.4.4). The degeneration of the tubules was observed at cranial to periphery of the pronephros at 25 to 27 days of gestation (1.5cm CRL to 1.7cm CRL). In 40 days of gestation (3.7cm CRL) at the level of 12^{th}-13^{th} vertebrae a stalk of degenerated pronephros was attached to the cranial pole of the mesonephros (Fig.12).

Table 4.3 Table showing micrometrical details of Pronephros

Sl.No	Gestational age (days)	Capsular space(µm)	Glomerular diameter (µm)	Tubules	
				Width (µm)	Length (µm)
1	20	2.5	225±1.3	25±1.2	105±1.6
2	24	7.5	193.7±2	55±1.2	95.6±1.7
3	25	10	150±1.6	31.2±1.5	62.5±1.5
4	27	15	75±1.8	29.3±1.6	48.1±1.9

Fig.4.1: Graph showing micrometrical details of Pronephros



4.2.2 Mesonephros

The mesonephros was established on the portion of pronephric duct, immediately behind the pronephric tubules. In 20 day (0.7cm CRL) old embryos the mesonephros was globular in shape and occupied the dorsal part of coelomic cavity from below the level of somites, cranially adjoined to the pronephros by primary excretory duct. The mesonephros was extended ventro-laterally in the coelomic cavity on either side of the aorta and ventro-medially related to the archenteron. The mesonephros was surrounded by many layers of mesenchymal cells which were interspersed by connective tissue fibers (Fig.13).

The mesonephric parenchyma was constituted with peripherally located glomeruli and centrally placed tubules unlike pronephros in 20 days sheep embryo. The parenchyma was densely packed with tubules, migrating angioblasts and differentiating glomerulus (Fig.13A). At 24 days (1.1cm CRL) the shape of the mesonephros was little elongated and connected medially to the gonads by a stalk. The mesonephros was surrounded by a thin capsule of 14±1.2 µm consisted of connective tissue fibers. The mesonephric parenchyma was differentiated into glomeruli, tubules and left over with scanty differentiating mesenchymal cells at the

posterior border of mesonephros. Angiogenic mesenchyme /angioblast cells were traversing in between the tubules, periphery of parenchyma consisted of large blood capillary (Fig.14).

At 25 days (1.5cm CRL) of gestation, the thickness of capsule was increased to 17±1.4 µm, without elastic fibers in it and the blood supply from the aorta was established predominantly to the mesonephric glomeruli. Posterior cardinal vein extended into mesonephros anteriorly over the tubules and ventrally mesonephros consisted of Wolffian duct (Fig.15&16). Thickness of the capsule was increased to 23±1.4µm. The number of glomerulus and tubules was drastically increased in 27 day old sheep embryo (1.7 cm CRL) (Fig.17). Between 29 to 31 days of gestation, (2 cm CRL) mesonephros related to the 7-8th somite cranially and extended upto the 16th somite caudally. The shape of the mesonephros was changed from globular to elongated/shuttle and covered with a thick capsule of 32±1.5µm in 37 to 39.5 days (3.5cm CRL) than the earlier age. The parenchyma was densely packed with many glomeruli and tubules (Fig.18).

In the embryo of 39.5 days (3.5cm CRL) the mesonephros prolonged to 18th somite in the coelomic cavity. The degenerative process was initiated in the anterior pole at this age. Typical elongated shuttle shaped mesonephros was present medial to the metanephric kidney by showing medial concave and lateral convex surfaces (Fig.19). At 49 days (7cm CRL) of gestation, the mesonephros was surrounded by a thick capsule consisted of connective tissue fibers. In the parenchyma, at the anterior pole the degenerative process was progressively increased than the earlier ages and the concavity towards the medial border was increased which accommodated testes into it (Fig.20).

At 57 days of gestation (10cm CRL), the space between the testes and the mesonephros was reduced than earlier age. The mesonephros was closely adherent to the testes and parenchyma was replaced with connective tissue (Fig.21). At 74 days of gestation, the mesonephros was covered by thick capsule which was lined by squamous cells and become as ductus deferens of the testes in this age (Fig.22)

4.2.2.1 Mesonephric glomeruli

In 20 days (0.7cm CRL) the mesonephros showed giant glomeruli, each were filled nearly whole of the Bowman's cup by leaving a small narrow space. The Bowman's capsule was lined by squamous to cuboidal cells. Large groups of mesenchymal cells and angioblasts, between the giant glomeruli were seen in ventro-caudal zone (Fig.13A & 23). At 24 and 25 days (1.1 to 1.5mm CRL), noticeable difference over that of the preceding stage. Both the cranial and caudal parts of mesonephros were differentiated and Bowman's/ capsular space were clearly noticed. The width of the capsular space was 14±1.2µm. The glomerular mass with tuft of capillaries filled the whole cavity of the Bowman's capsules. The average size of the glomerulus was 210±2.4µm (Table 4.4). Glomeruli surrounding visceral and parietal layers were demarcated at this age. The parietal layer and visceral layers were lined by simple squamous epithelium. In the visceral layer, between the squamous cells very few cells were modified and presented with large vesiculated nucleus, representing the future podocytes. Collagen fibers were interspersed between the tuft of glomeruli (Fig.14 & 24). Anterior pole of the glomeruli showed reticulocytes on one side and developing tubules on the other side. Individual transverse branches from the primitive aorta were passed along close to the mesonephros to the glomerulus was began at this age. The glomerular columns in the middle zone

converged dorso-medially to join the mesonephric tubules at the dorsal end and the glomeruli number was increased in caudal zone (Fig.14, 15&24).

The manifestation of macula densa of DCT, Juxta glomerular cells of afferent arteriole and mesengial cells of smooth muscle at vascular pole of Bowman's capsule was observed in 27 days foetus (1.7 cm CRL) (Fig.25). In 35.5 days sheep embryo (3 cm CRL), the glomeruli were centrally placed in the mesonephros and were oriented in a linear fashion, which extended from the cranio-medial to the caudal pole of mesonephros. The glomeruli appeared as many lobules with basal and apical connections at this age.

The degenerative changes were advanced in cranio-caudal border to middle and adjoining 1/3rd part of the caudal zone of mesonephros in embryos of 39.5 days (3.5cm CRL). The mesonephric glomeruli lost their epithelial organization and the size was reduced. The relative size of the glomeruli decreased over that in embryos of preceding group. Their fragmentation and shrinkage caused a noticeable space between glomerular mass and the Bowman's capsule. The degenerative changes further advanced in sheep embryo of later series (Fig.19).

In 49 days (7 cm CRL) of gestation, the glomerulus shrinkage was noticed and the parietal layer of glomerulus was thickened with increased capsular space (Fig.20). In 50 days foetus (7.5 cm CRL) the relative number of glomerulus was further reduced, in the parietal layer at urinary pole, the cells of Bowman's capsule were slowly transformed into cuboidal cells with increased capsular space (Fig.26). At 57 days (10 cm CRL) of gestation the fragmented glomeruli with indistinct visceral layer and increased Bowman's space could be observed in whole mesonephros (Fig.21).

4.2.2.2 Mesonephric tubules

Sheep embryos of 20 days (0.7cm CRL) showed many of irregular tubules, which were differentiated through the condensation of mesenchymal cells, some were epithelialized and formed vesicles with narrow and wide lumen in the parenchyma of mesonephros. The tubules with narrow lumen were lined with simple cuboidal epithelial cells and filled with eosinophilic material in the lumen, while some of them were presented without any evidence of substance and showed wide lumen. These tubules were surrounding the glomeruli. Some of the tubules were closely related to the anterior and posterior poles of glomeruli. The cranial and caudal zones of mesonephros were possessed with clusters of eosinophilic angiogenic mesenchymal cells which were progressively differentiating into PCT. The periphery of the mesonephric parenchyma consisted of well-defined vesicles, many of which showed ventral elongation as progenitors of tubules. These were usually straight, lined with simple cuboidal epithelium of uniform height, wide lumen and without brush borders (Fig.13A).

In 24 days embryo (1.1cm CRL) the mesonephric tubules were grown in length than the earlier age (Table 4.5), took an elongated /comma shaped curves. Tubules with thin wall and wide lumen were appeared at the periphery of mesonephros, which were lined by cuboidal cells. Most of its tubules established continuity with the existing mesonephric duct. The angiogenic cells at places reorganized themselves as capillaries. The relative number and size of the mesonephric tubules were increased than in previous gestation period. Some tubules with wider lumen lined by cuboidal cells with basally placed nuclei and their cytoplasm showed foamy appearance with eosin. Most of the tubules in the parenchyma showed histological differentiation into the proximal and distal segments. Between the tubules nucleated blood cells were noticed in the parenchyma (Fig.14).

The proximal and distal segments of mesonephric tubules were sharply defined in sheep embryos of 25 day (1.5 cm CRL). The lumen of proximal tubules showed an esinophilic material. The mesonephric duct located at the periphery in the postero-lateral zone of the mesonephros, which was lined by cuboidal cells. The middle and caudal zones revealed no organizational changes over the preceding stage (Fig.15). At 27 days (1.7cm CRL) the tubular lumens showed eosinophlic material with increased in the size of the tubule and at the periphery the size of ducts were increased and possessed wide lumen compare to earlier stages. The mesonephric duct presented at the caudal aspect of the gland (Fig.15).

In 31 days (2cm CRL) the proximal and distal segments of mesonephric tubules were easily identifiable based on the differences in height, staining affinities and their epithelial cells. The proximal tubules possessed narrow lumen with brush borders. Thin walled irregular sized collecting tubules were noticed at the periphery of the gland. At 35.5 days (3cm CRL) the $3/4^{th}$ of parenchyma was occupied by densely packed tubules presented towards the periphery of mesonephros were irregular in shape, some of them were lined by cuboidal cells with narrow lumen, and some others were also lined by squamous to cuboidal cells with wide lumen. At this age there were striking similarities in structure between the mesonephros and metanephros but there was no demarcation of cortex and medulla and lack of loop of Henle.

The structural distortion of mesonephric tubules marked the onset of degenerative process and atrophied in the cranial zone of the mesonephros in 39.5 days embryo (3.5cm CRL). The cranial tubules lost their epithelial cell lining and stained more intensely. In the middle zone, the tubules were reduced in size, some tubules lost their epithelium and the caudal tubules were still developing. (Fig.19).

The degenerative changes in 44 days (5cm CRL) indicated by disappearance of epithelial organization in the tubules. The concentration of the tubules was decreased at 46.5 days (6 cm CRL) and increased the size of the capsule.

Complete cellular disintegration of tubules was notified at 1/3rd of the cranial mesonephros and the 2/3rd tubules were in degenerating process with small vacuolations in the tubular cells at 49 days (7cm CRL) (Fig.20). In 57 to 59 days (10cm to 12cm CRL) 3 to 4 mesonephric tubules were persisted at the cranial end as small round to spherical tubules with very narrow lumen and were lined by cuboidal cells, as aberrant ductules, the succeeding 7-8 tubules were little larger than the preceding. (Fig.21). In 74 days (16cm CRL) foetuses,12-13 tubules in cranial part towards testes were round to elongated, lined by cuboidal cells with narrow lumen, develop into efferent ductules of testes followed which the tubules were rounded with little wider lumen than preceding ones, future head of the epididymis (Fig. 22).

Fig. 4.2 Bar diagram showing micrometrical details of Mesonephros

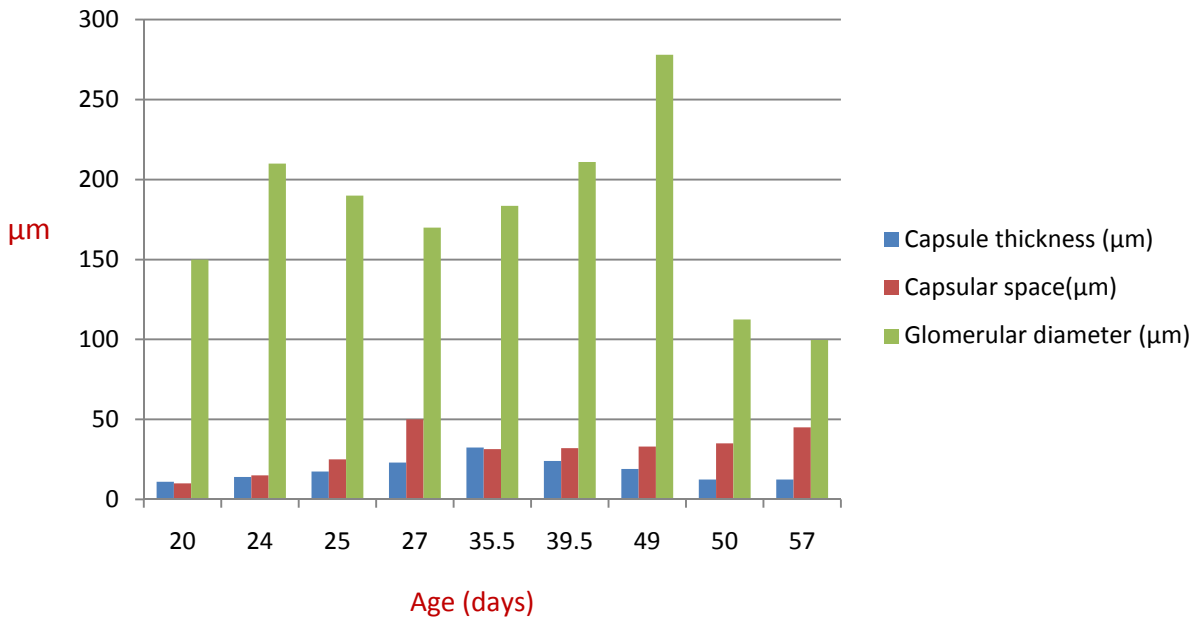


Fig. 4.2a Bar diagram showing micrometrical details of Mesonephros

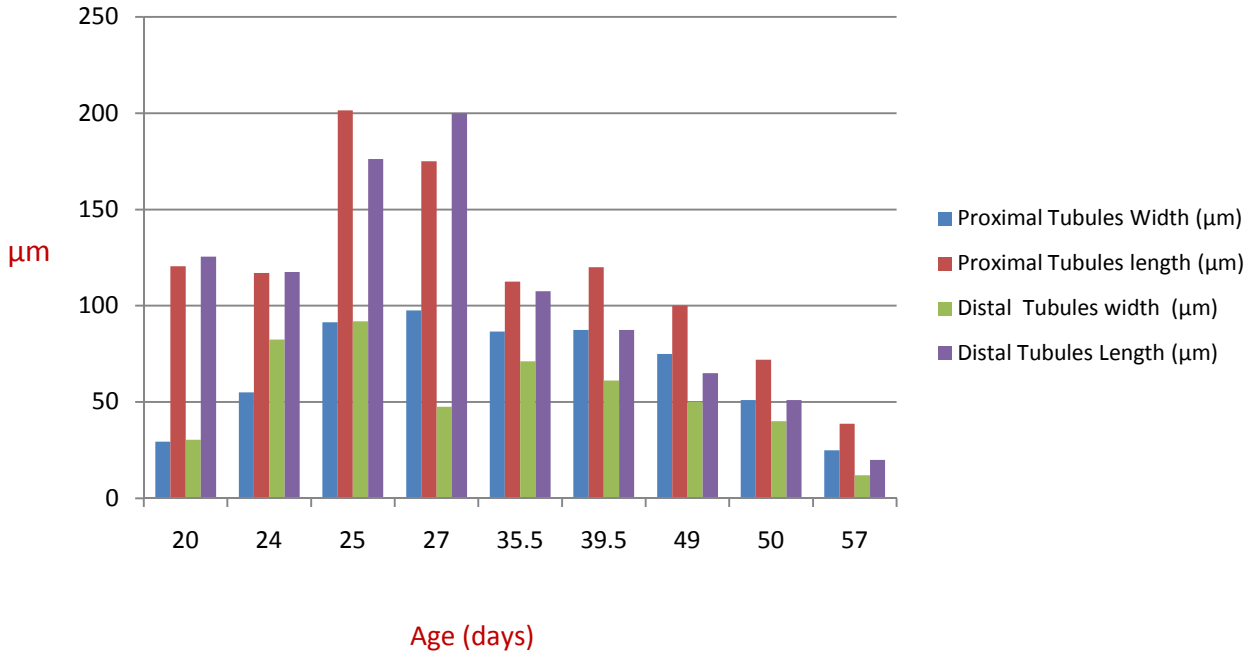


Table 4.4 Showing micrometrical details of the mesonephros (Mean ± Standard error) in various gestations

Sl.No	Age in days	Capsular thickness (µm)	Capsular space(µm)	Glomerular diameter (µm)	Proximal Tubules		Distal tubules	
					Width (µm)	Length (µm)	Width (µm)	Length (µm)
1	20	11±1.1	10±1.2	150±2.3	29±2.4	120±1.0	30±3.2	125±2.3
2	24	14±1.2	15±1.3	210±2.4	55±2.6	117±2.5	82±3.3	117±2.5
3	25	17±1.4	25±1.7	190±3.1	91±2.7	203±3.8	92±2.5	176±2.5
4	27	23±1.4	50±1.4	170±2.6	97±3.5	175±3.2	47±2.5	200±4.2
5	35.5	32±1.5	31.±1.7	183±2.6	86±4.5	112±2.6	71±2.6	107±6.2
6	39.5	24±1.6	32±2.1	211±2.4	87±5.5	120±2.4	61±2.4	87±7.2
7	49	19±2.5	33±2.5	278±2.9	75±5.6	100±5.6	50±2.9	65±3.2
8	50	12±2.4	35±2.7	112±3.4	51±5.7	72±2.5	40±2.6	51 ±1.8
9	57	12±2.6	45±2.1	99±3.4	25±2.4	38.±2.9	12±4.2	20±1.6

4.2.3 Metanephric kidney

4.2.3.1 Ureteric bud

The ureteric bud was formed by evagination of the caudal part of the mesonephric duct in sheep embryo at 20 days of gestation (0.7 cm CRL). The future duct system of metanephros was developed from this ureteric bud. The epithelial cells lined the primordial duct stained lightly with eosin, the basally placed nuclei were highly basophilic and cells were arranged in 2-3 layers. This bud possessed a very narrow lumen. The ureteric bud surrounded by a mass of mesenchymal cells (Fig. 27). The ureteric bud elongated cranially, the blind end was ampullated and capped by metanephric blastemal cells in 24 days embryos (1.1 cm CRL). At this stage, the ureteric bud possessed a wide lumen compared to the earlier age, which placed on either side of the dorsal aorta. Angiogenic mesenchyme migrates slightly into the metanephric blastema to produce the glomeruli and vasa recta (Fig. 28)

During 25 to 30 days of gestation (2 cm CRL) the ureteric bud undergone a series of bifurcations within the surrounding metanephric mesenchyme, and formed varied number of smaller ureteric ducts. At the same time the metanephric mesenchyme condensed around the dividing ducts and formed S-shaped clusters, which transformed into epithelia and fused at their distal ends with the ureteric ducts. The branching was completed and the metanephric blastema was fully transformed to rounded cap like structures and primitive tubules. The relative density of cell mass was increased gradually with advanced age. The primitive tubules were lined by cuboidal cells, with eosinophilic cytoplasm and basophilic nuclei (Fig. 29 & 30).

4.2.3.4 Metaneprhric blastema

Between 25 to 29 days, the mesenchymal clusters were thus converted to small groups of epithelial cells, which undergone complex morphogenic changes.

Each epithelial group elongated, to form comma-shaped, then an S-shaped, body, which continued and fused at its distal end with a branch of ureteric duct and expanded as a dilated sac at its proximal end. The blood vessels invaded the proximal ends of the S-shaped clusters to form vascularized glomeruli (Fig. 28, 29 & 30). Thus the developing renal corpuscle was noticed. The ureter emerged out from the hilus of the kidney at this age was established. The mesenchymal cells bordering whole of the nephrogenic mass condensed at periphery as primordial capsule of the developing metanephric kidney in 29 days of embryos (1.9 cm CRL) (Fig.18). The para-cortical region consisted of 2 to 3 glomeruli were differentiated with capsular space in 36 days embryo (2.5cm CRL).

4.2.3.5 Renal corpuscle

At 37.5 days embryo (3cm CRL) metanephric kidney was surrounded by the undifferentiated mesenchymal cell mass which invading from periphery to the centre between the tubules at periphery, future lobules and pyramids of the kidney. There was no demarcation as cortex and medulla at this age. The number of glomerular masses was increased than earlier ages, which were suspended between the tubules and at the centre of the gland. Some renal corpuscle, showed glomerulus with clear Bowman's capsule. The nephrogenic mass surrounding glomerulus was differentiated and modified as parietal and visceral layer of capsular cells surrounding capillary network (Fig.31).

Metanephric kidney was divided into dark coloured cortex and lighter colour medulla at 44 days (5cm CRL) of gestation. The renal corpuscles were distributed between the para cortical and juxta-medullary areas. The size of the renal corpuscle was 75±1.6µm. Few renal columns of Bertini were noticed at this age. Each renal corpuscle showed a tuft of glomerular capillaries and Bowman's space was evident,

parietal layer was lined with squamous cells and the visceral layer closely adhered to the glomerular network as podocyte like cells. At the vascular pole of the glomerulus distal convoluted tubules were identified but no evidence of juxta glomerular cells in this age group (Fig.32&33).

The number of the renal corpuscles was increased. Renal corpuscles were distributed towards periphery and 2-4 corpuscles were obvious towards future apex of each pyramid where they are particularly related to juxta medullary regions of the parenchyma. Internal lobulation was evident in the cranial region of kidney at 50 days (7.5cm CRL) of gestation. Renal corpuscles were surrounded by parietal squamous to cuboidal cells, visceral layer was closely adhered to glomerular endothelium and lined by podocytes. The size of the capsular space was increased to 80±5.3µm up to 57days (10cm CRL). Between the endothelial cells some cells were modified as mesangial cells. Vascular pole of renal corpuscles were more evident with blood supply into it. But no evidence of juxta Glomerular complex cells (Fig. 34, 35 & 36). Renal corpuscles were occupied at intermediate zone in 71 days (Fig. 37). At 78 days, the capsular space was 12±2.1µm, reduced than the earlier ages (Table.4.5). The PCT was lined by cuboidal cells with brush border in their lumen. At few corpuscles slight modification of cells was observed in DCT. Many of the corpuscles were traversing towards cortex than previous ages (Fig.38). At 96 days, Juxta glomerular apparatus cells were well developed in the intermediate nephrons (Fig.39).

In group III the typical cortex and medulla was differentiated as adult kidney and renal corpuscles were still under differentiating stages in the cortical region by 101 days. Capsular space was decreased than earlier age (Table 4.5). Juxta glomerular complex was present (Fig.40). At 107 days, the cortex was clearly differentiated into cortical labrynth and medullary rays. Renal cropuscles differentiation was progressed

than previous age. Capsular space was surrounded by parietal squamous cell and visceral podocytes and basement membranes of the renal corpuscle were well demarcated by 115 days (Fig.41 & 42). By 120 days Juxta glomerular cells exhibited the cytoplasmic granules (Fig.43). Indicated the function status of kidney. At 144 days, histologicaly kidney was well differentiated as adult kidney and the cortical nephrons/renal corpuscles were distributed throught the cortex (Fig. 44)

4.2.3.6 Metanephric tubules

At 37.5 days the metanephric tubules were occupied throught the periphery and centre. The tubules at periphery were lined by cuboidal cells with narrow lumen, but at the centre tubules were with wide lumen (Fig.31).The metanephros was surrounded by a capsule with many layers of connective tissue fibers and spindle shaped nucleated cells. Demarcation between proximal and distal tubules was noticed in 44 days of gestation (Fig.32&33).

Sheep foetus of 50 days showed increased tubular density than earlier age. Most of the mesenchymal mass in the parenchyma of the gland was differentiated into tubules. In between the renal corpsules collecting tubules were present towards the apex in some of the pyramids. Renal pyramids were not apparent at this age except in the caudal aspect, at one place of the caudal region medullary rays were extended from apical zone of the renal pyramid to the cortical area of the metanphric kidney. Increased quantity of collagen fibers at the sinus was noticed than earlier ages but elastic fibers were not noticed and prominent renal sinus was noticed at 50 days (Fig. 34&35). Between 50 to 60 days the tubules around the renal corpuscles were modified into proximal and distal tubules, which were present in the close proximity of it. Proximal tubules were lined by irregularly oriented flat to cuboidal cells with eosinophilic cytoplasm and distal tubules were regularly arranged cuboidal cells with

wide lumen. Though they were closely adhered to corpuscles, the cells were not modified into macula densa cells and Loop of Henle were not evident upto this age. But around the renal corpuscles cluster of collecting tublues were prominently seen at this age.

Major and minor calyces were apparent at 57 to 60 days of gestation and increased quantity of collagen at the sinus around the major and minor calyx and also between and around the tubules in the cortical region. Towards renal sinus the parenchyma was gradually modifying into pyramid like structure whose epicss were facing into developing minor calyces. In between these pyramid the parenchyma was projected as columns of Bertini (Fig.35). Renal columns were more evident in 71 days than earlier age. The papillae opened into minor calyces. Some minor calyx were coalesced together to form major calyces at this age (Fig.37). Loop of Henle was first appeared at 83.5 days (19.5cm CRL), which were well established in juxta medullary nephrons as descending and ascending limbs by 96 days and the renal columns of Bertni were well established by which renal pyramids were more obvious. Macula densa cells were identified in juxta medullary nephrons at this age. Nerve fibers were evident between the tubules in the medulla from 96 days onwards (Fig.39&46). Papillary ducts were lined by stratified cuboidal epithelium (Fig.47). Minor calyx were lined by transitional epithelium like adult kidney and smooth muscles were also noticed in the wall of the calyx minor in 115 days of gestation (Fig.48).

4.2.3.2 Metanephric capsule

At 30 to 37 days (2 cm CRL), the mesenchymal cells bordering the gland were modified to form thin layer of capsule, which was eosinophilic with few connective tissue fibers, mainly collagen with fibroblasts and few reticular fibers especially in 35.5 days (3 cm CRL). In 45 days of gestation (5.5 cm CRL) the capsule thickness

was increased and lined by single layer of squamous cells with few nerve fibers. The collagen fibers were arranged in thick bundles with increased capsular thickness in 50 days (7.5cm CRL) and in addition to which increased elastic fibre content in the capsule of 57 days embryos (10cm CRL) (Fig.33). The thickness further increased with wavy bundles of collagen fibers with a thin layer of elastic fibers interspersed in 78 days of gastation (17.5cm CRL) (Fig.35). Collagen fibers were arranged in wavy manner with spindle shaped fibroblast with increased quantity of reticular fibers in 83.5 days (19.5cm CRL) of gestation (Table 4.6).Very few collagen fibers were found at 90days .Many reticular fibers were present by 107 days.

4.3 Histochemisrty

4.3.1 Neutral Mucopolysaccharides

In group I at 44 days (5cm CRL) renal corpuscles and tubules showed PAS-negative reaction. In Group II at 96 days (24cm CRL) there was a mild reaction in the epithelial cells lining the ducts and tubules at luminal surface. In group III at 101 days (26cm CRL) the capsule and tubules showed mild PAS- positive reaction and the cytoplasm of collecting ducts showed intense PAS- positive reaction indicating the neutral mucopolysccharides in the collecting ducts. Basement membranes of the renal corpuscle, tubules and collecting ducts were positive for PAS (Fig. 49).

4.3.2 Alkaline phosphatases

In group I to group III the renal corpuscles, tubules and collecting ducts showed negative reaction for both acid and alkaline phosphatases (Fig. 50).

Table 4.5 Showing micrometrical details of the Metanephros (Mean ± Standard error) in various gestational periods.

Sl.No	Age (days)	Capsule thickness (µm)	Capsular space (µm)	Glomerular diameter (µm)	Distal Tubules		Proximal tubules	
					Width (µm)	Length (µm)	Width (µm)	Length (µm)
1	31	5±0.4	7.5±0.2	87±2.3	56±2.3	81± 3.5	58±5.2	106± 2.6
2	41	10±0.2	12.5±12	89±1.3	50±5.3	62± 4.8	68±4.6	112±4.6
3	44	15±1.5	12.5±2.3	75±1.6	35±5.6	66±4.5	81±4.5	135±4.2
4	46.5	15± 1.3	13±1.3	75±1.6	32±5.7	65±3.6	85±8	127±11
5	50	16±1.6	12±1.2	75±4.5	25±6	65±5	75±9	115±10
6	57	17±2.1.7	12±1.6	80±5.3	31±5.6	62±6	50±6.5	112±12
7	60	17±1.1	12±1.5	50±5.9	29±7	51±7	53±3.2	95±9
8	78	25±1.2	12±2.1	50±2.4	28±5.6	50±6	50±4.2	100±12
9	83.5	25±1..3	12±2.8	40±5.6	27±8	50±5.7	50±4.3	75±5
10	87	26±1.0	12±2.6	37±6.4	25±5.8	50±5.7	50±4.3	75±5
11	96	27±1.3	12±3.5	35±5.2	25±3.6	50±57	50±4.3	74±5
12	101	29±1.3	10±2.6	35±6.1	20±8	50±5.7	50±4.3	74±5
13	107	29±1.3	10±2.6	35±4.6	20±7	50±5.6	50±1.6	70±1.3
14	120	35±0.2	10±3.6	30±2.6	18±5.1	34±5.6	35±1.9	65±6.3
15	150	37±0.4	10±3.1	25±4.3	17±8	32±4.8	35± 4.4	62±1.6

Fig.4.3: Bar diagram showing micrometrical details of Metanephros

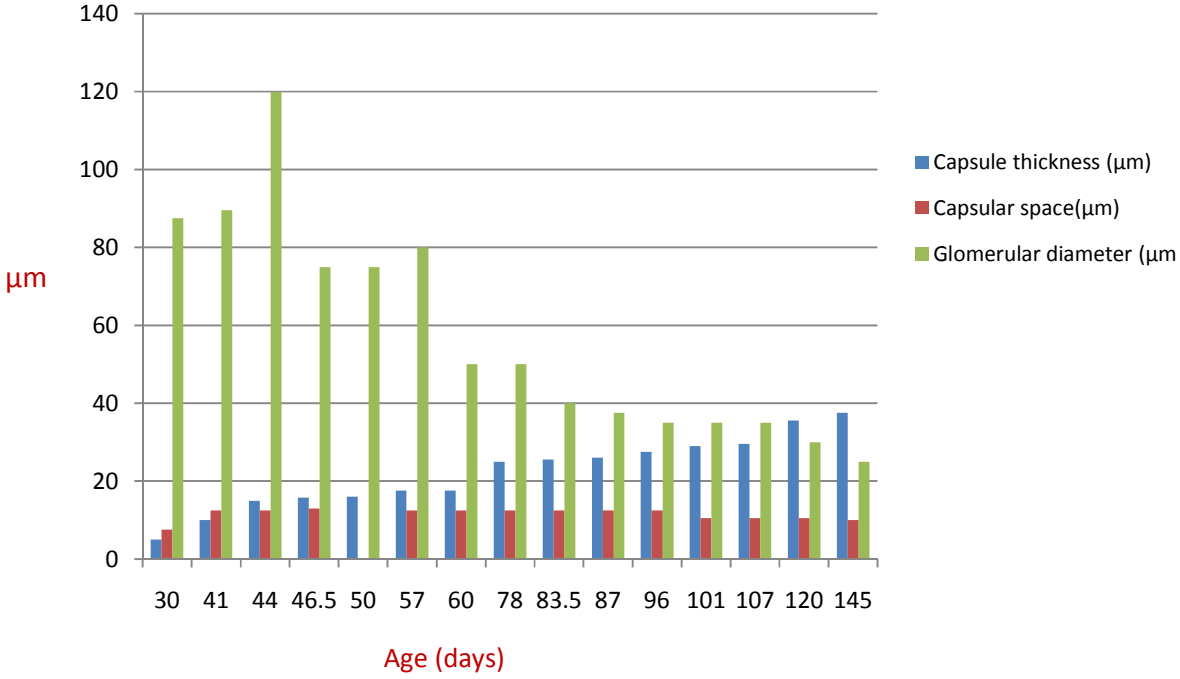
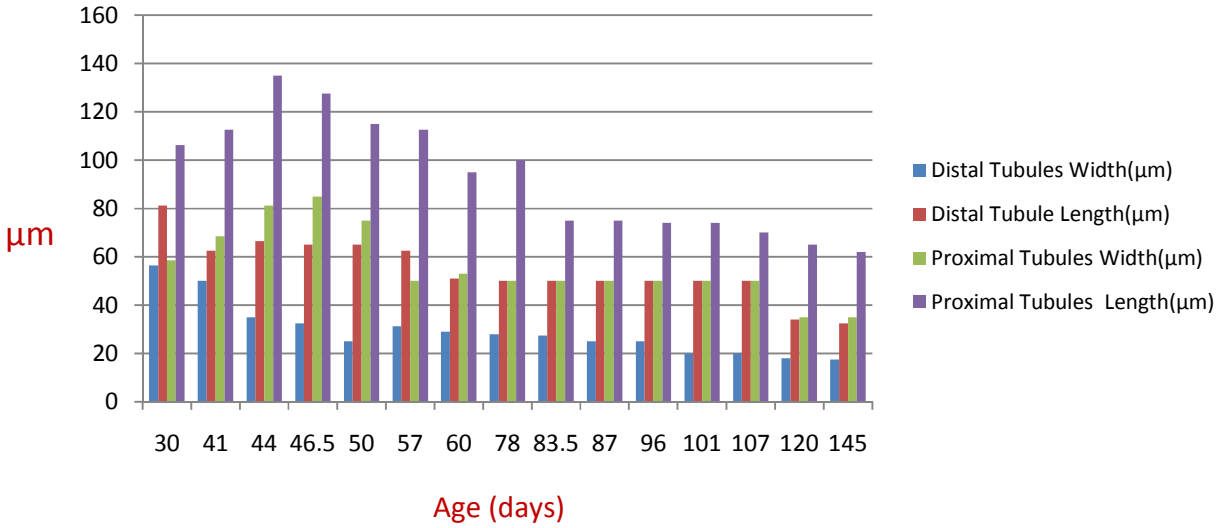
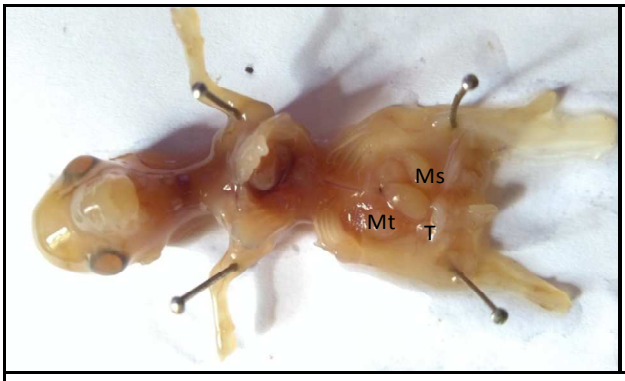


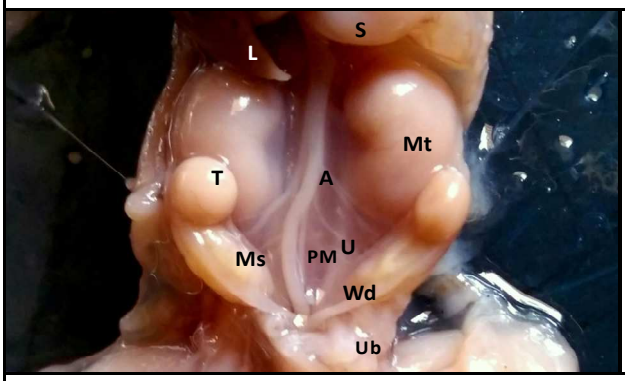
Fig.4.3a Bar diagram showing micrometrical details of Metanephric tubules





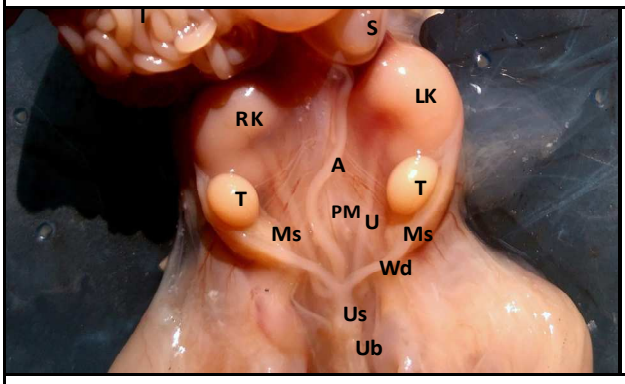
Ms-Mesonephros
Mt-Metanephros
T-Testes

Fig.1 Photograph of 39 day old sheep embryo showing the location of kidneys



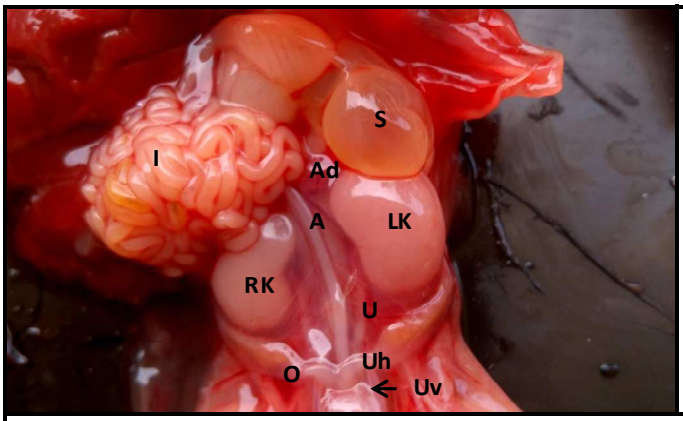
Ms-Mesonephros
Mt-Metanephros
T-Testes
A- Aorta
U- Ureter
S- Stomach
L-Liver
Wd-Wolffian duct
PM-Psoas Major
Ub-Urinary bladder

Fig.2 Photograph of 41 day old sheep embryo showing the location of kidneys



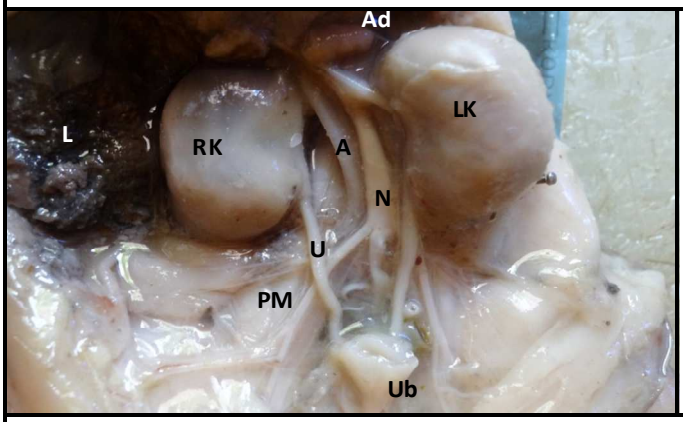
Ms-Mesonephros
LK-Left Kidney
RK-Right Kidney
T-Testes
A- Aorta
U- Ureter
S-Stomach
Us-Urogenital sinus
Wd-Wolffian duct
I-Intestines
PM-Psoas Major
Ub-Urinary bladder

Fig.3 Photograph of 50 day old sheep embryo showing the location of kidneys.



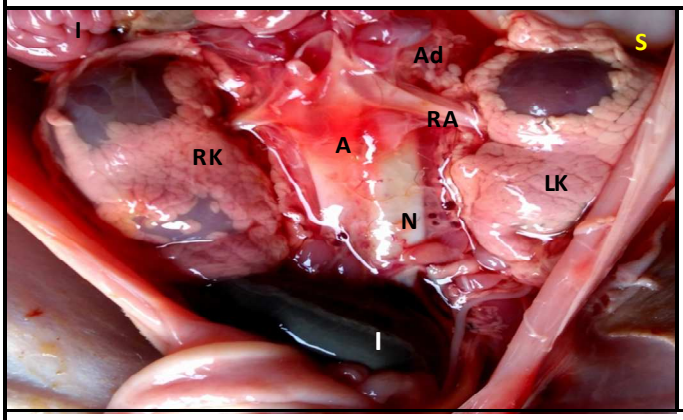
LK-Left Kidney
RK-Right Kidney
Ad- Adrenal gland
O-Ovary
A- Aorta
U- Ureter
S-Stomach
Uh-Uterine horns
I-Intestines
Uv-Uterovesical pouch

Fig. 4 Photograph of 60 day old foetus showing the location of kidneys.



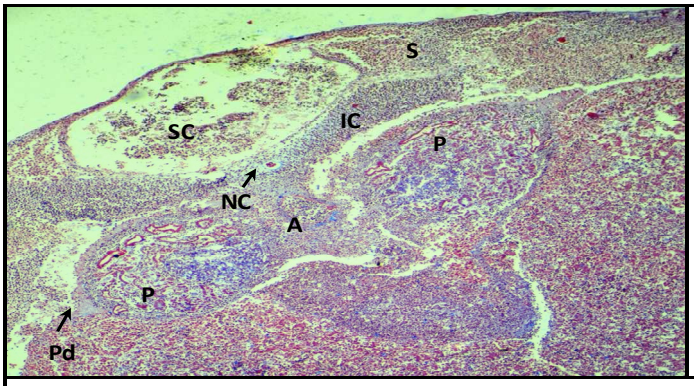
LK-Left Kidney
RK-Right Kidney
Ad- Adrenal gland
A- Aorta
U- Ureter
L-Liver
N- Nerve plexus
Ub-Urinary bladder
PM-Psoas Major

Fig. 5 Photograph of 78 day old foetus showing the metanephric kidneys.



LK-Left Kidney
RK-Right Kidney
Ad- Adrenal gland
A- Aorta
RA-Renal artery
U- Ureter
S-Stomach
I-Intestines
N-Nerve

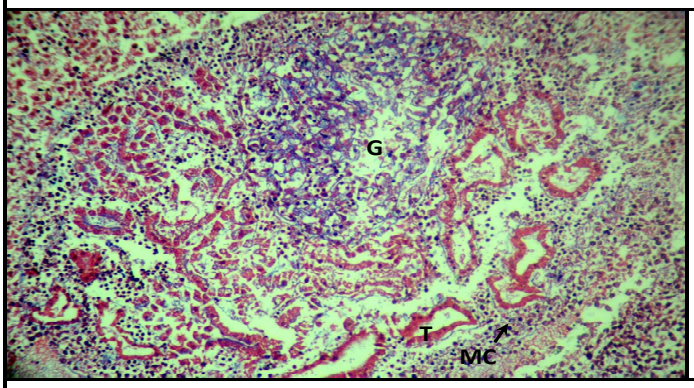
Fig. 6 Photograph of 145 day old foetus showing the metanephric kidneys.



S-Somite
SC-Spinal cord
IC-Intermediate cell mass
NC-Notocord
A-Dorsal aorta
P-Pronephros
Pd-Pronephric duct

Fig.7 Photomicrograph of 20 day old sheep embryo showing pronephros.

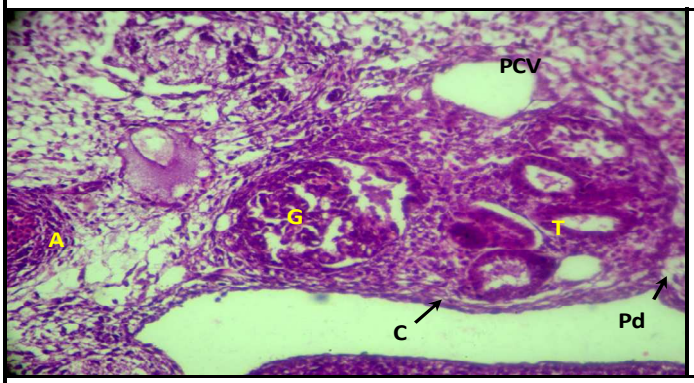
Masson'sTrichromeX100



G- Gaint Glomeruls
T-Tubules
MC- Mesenchymal cells.

Fig.8 Photomicrograph of 20 day old sheep embryo showing pronephric glomeruli.

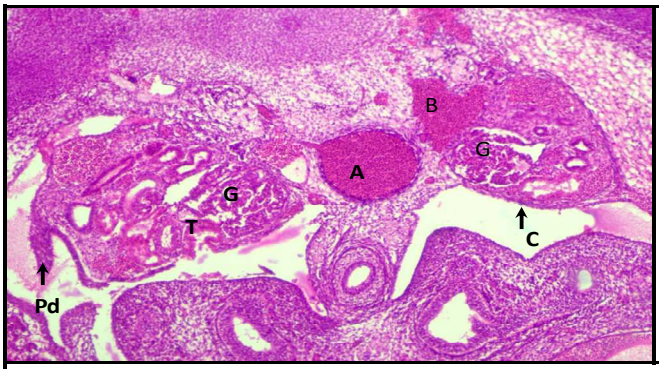
Masson'sTrichromeX400



A-Aorta
G-Giant Glomerulus
T-Tubules
PCV- Posterior cardinal vein
C-Capsule
Pd-Pronephric duct

Fig.9 Photomicrograph of 24 day old sheep embryo showing pronephric glomeruli.

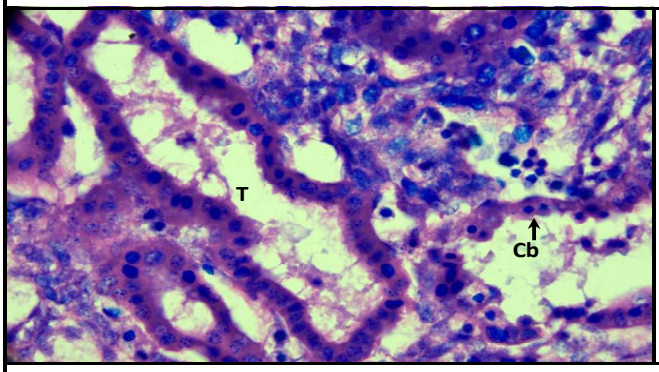
H&E X400



B-Blood islands
G- Gaint glomeruls
T-Tubules
A-Aorta
C-Capsule
Pd-Pronephric duct

Fig.10 Photomicrograph of 25 day old sheep embryo showing pronephros.

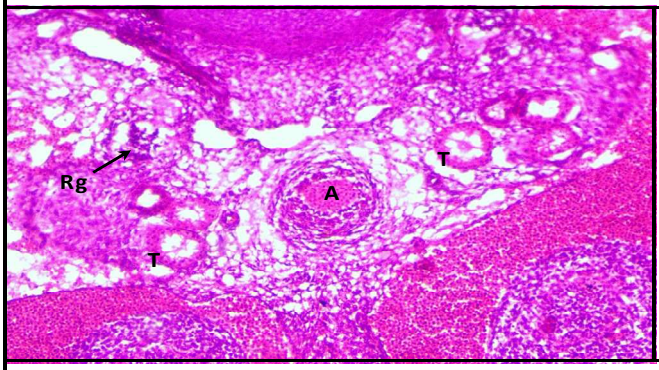
H&E X400



T-Tubules
Cb-Cuboidal cells

Fig.11 Photomicrograph of 25 day old sheep embryo showing pronephric tubules.

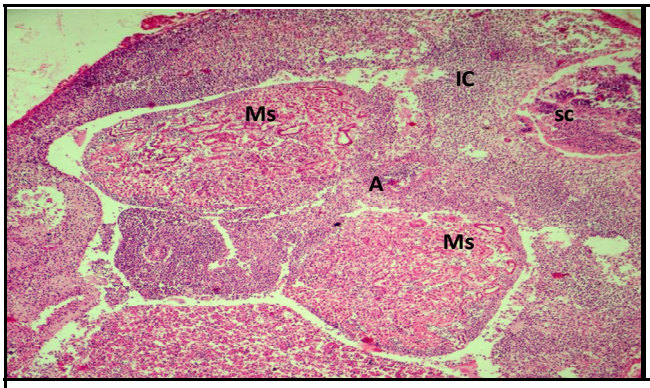
Luxal Fast Blue X1000



Rg- Remnants of glomerulus
A- Aorta
T-Tubule

Fig.12 Photomicrograph of 27 day old sheep embryo showing pronephric tubules.

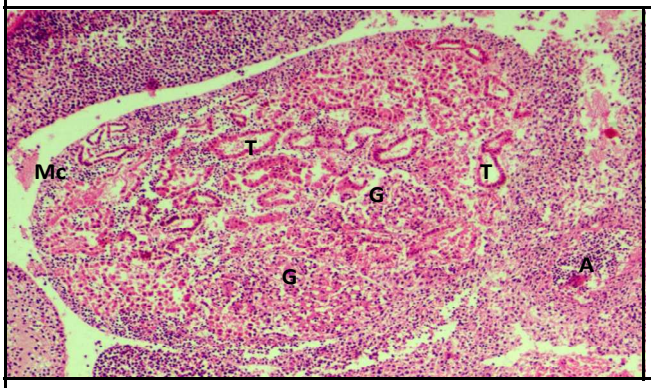
H&E X400



IC- Intermediate cell mass
Ms-Mesonephros
SC-Spinalcord
A-Aorta

Fig.13 Photomicrograph of 20 day old sheep embryo showing mesonephros.

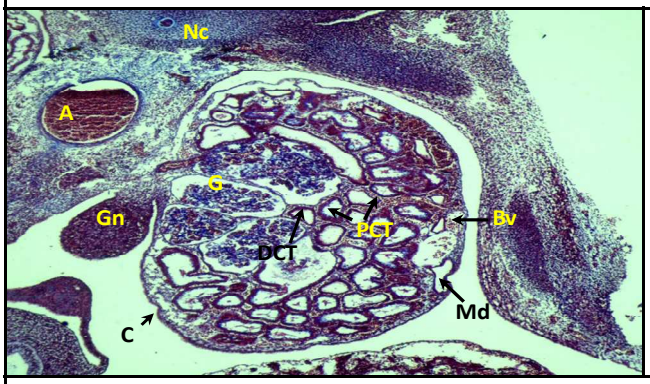
H&E X100



G-Glomeruli
T-Tubule
Mc-Mesenchymal Cells
A-Aorta

Fig.13 A Photomicrograph of 20 day old sheep embryo showing mesonephros.

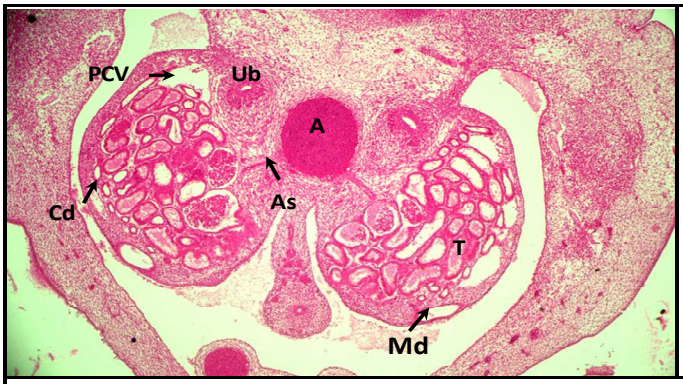
H&E X400



Nc-Notocord
A-Aorta
G-Gomeruli
Gn-Gonad
DCT-Distal Convoluted tubule
PCT- Proximal convoluted tuble
Md-Mesonephric duct

Fig.14 Photomicrograph of 24 day old sheep embryo showing mesonephros.

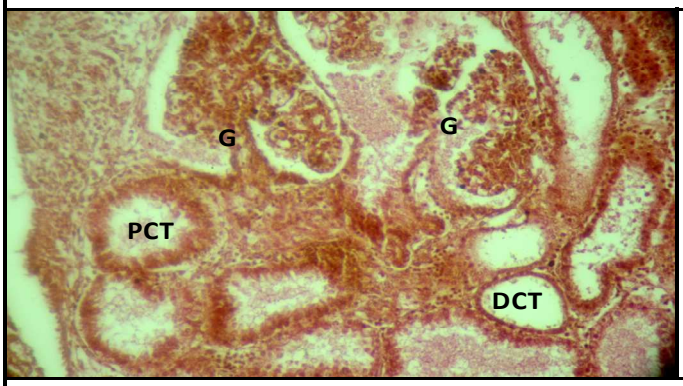
Masson's Trichrome X100



Ub-Ureteric bud
PCV- Posterior cardinal vein
A-Aorta
As- Arterial supply
T-tubules
Cd-Collecting ducts
Md-Mesonephric duct

Fig.15 Photomicrograph of 25 day old sheep embryo showing mesonephros.

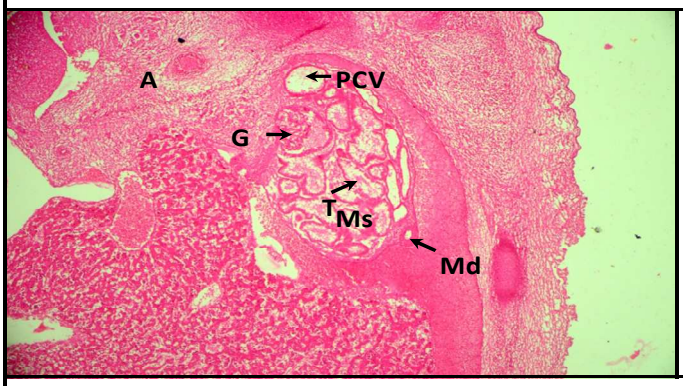
H&E X100



G-Glomerulus
PCT- Proximal convoluted tubule
DCT-Distal convoluted tubule

Fig.16 Photomicrograph of 25 day old sheep embryo showing mesonephros.

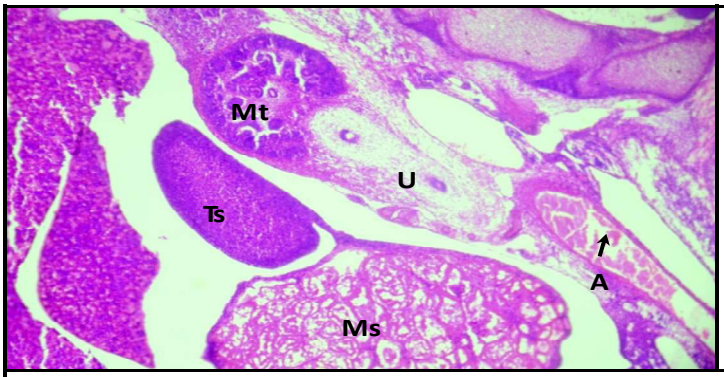
Verhoeff's X400



A- Aorta
G-Glomerulus
T-Tubules
PCV-Posterior cardinal vein
Ms-Mesonephros
Md-Mesonephric duct

Fig.17 Photomicrograph of 27 day old sheep embryo showing mesonephros.

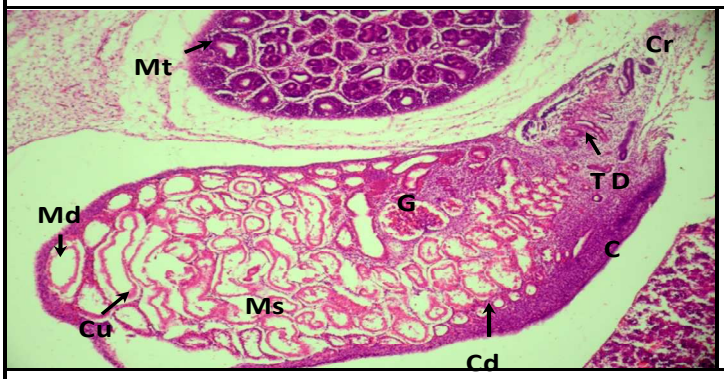
H&E X100



Mt- Metanephros
Ts-Testes
Ms-Mesonephros
A-Aorta
U-Ureter

Fig. 18 Photomicrograph of 29 day old sheep embryo showing mesonephros.

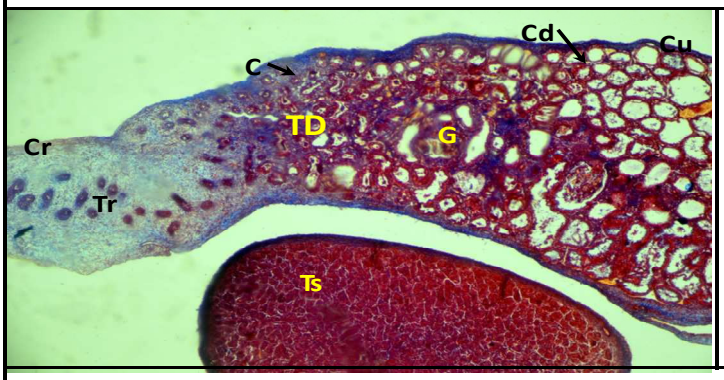
H&E X100



Mt- Metanephros
Md-Mesonephric duct
TD- Degenerating tubules
Cd-Collecting ducts
G-Glomeruli
C-Capsule
Cr- Cranial end
Cu- Caudal end

Fig.19 Photomicrograph of 39.5 day old sheep embryo showing mesonephros degeneration.

H&E X100



Cu-caudal end
Cr-Cranial end
C-capsule
Tr-Tubular remenants
TD-Degenerating tubules
G-Glomerulus
Ts-Testes

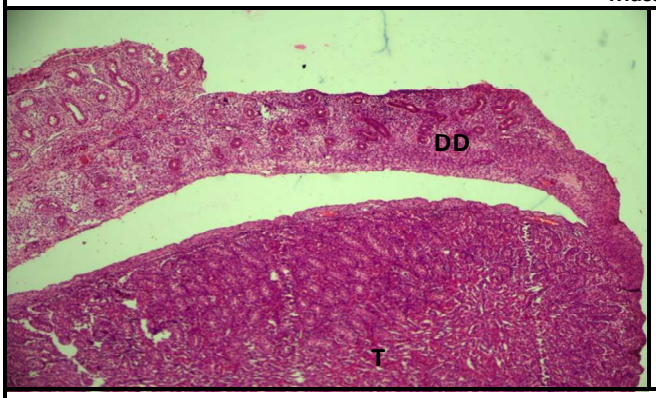
Fig.20 Photomicrograph of 49 day old embryo showing mesonephros degeneration.

Masson's trichromeX100

AD- Abbarent ductules
ED-Efferent ductules of the testes
Gd-Degenerating Glomeruli
T-Testes

Fig. 21 Photomicrograph of 57 day old sheep embryo showing mesonephros degeneration.

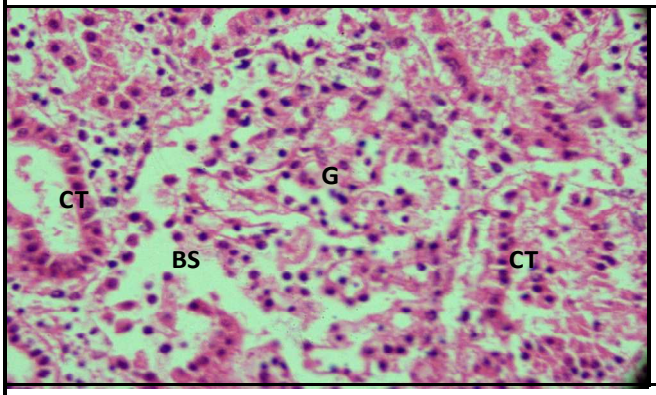
Masson's trichromeX100



DD- Ductus differense
T-Testes

Fig.22 Photomicrograph of 74 day old sheep foetus showing mesonephros transformation.

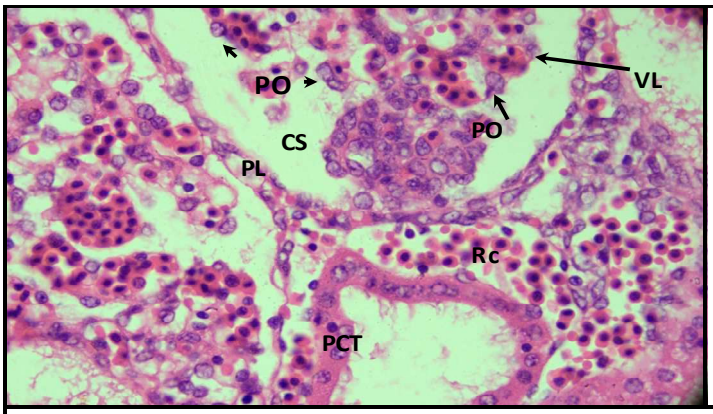
H&E X100



G-Glomerulus
CT- Convoluted tubule
BS-Bowman's Space

Fig.23 Photomicrograph of 20 day old sheep embryo showing mesonephros.

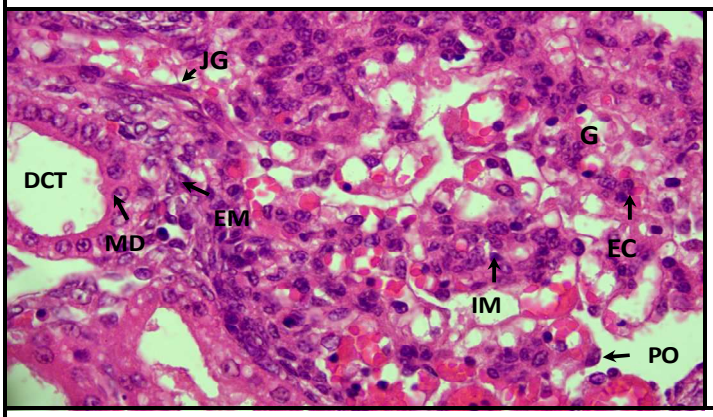
H&E X1000



VL-Viscerl layers
PO- Podocyte
PL-Parietal layer
CS- Bowman's space
PCT-Proximal convoulted tubules
Rc- Reticulocyte

Fig. 24 Photomicrograph of 24 day old sheep embryo showing mesonephric corpuscle

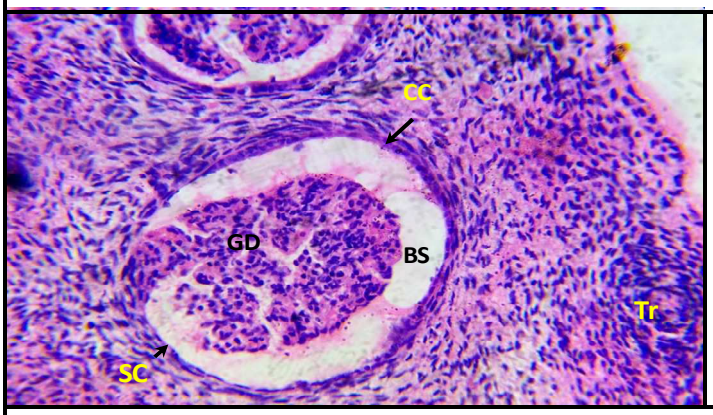
H&E X1000



JG-Juxta Glomerular cells
MD-Macula Densa cells
EM-Extra Mesengial cells
IM-Intra Mesengial cells
EC-Endothelial cells
PO-Podocytes

Fig. 25 Photomicrograph of 44 day old sheep embryo showing macula densa in mesonephros.

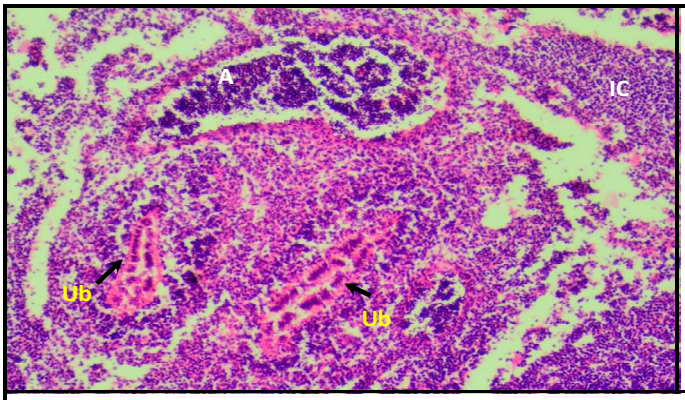
H&E X1000



GD- degenerating glomerulus
BS- Bowman's space
SC- Squamous cells
CC-Cuboidal cells
Tr-tubular remenant

Fig. 26 Photomicrograph of 50 day old sheep embryo showing glomerular degeneration.

H&E X400



IC-Intemediate cell mass
A- Aorta
Ub-Ureteric bud

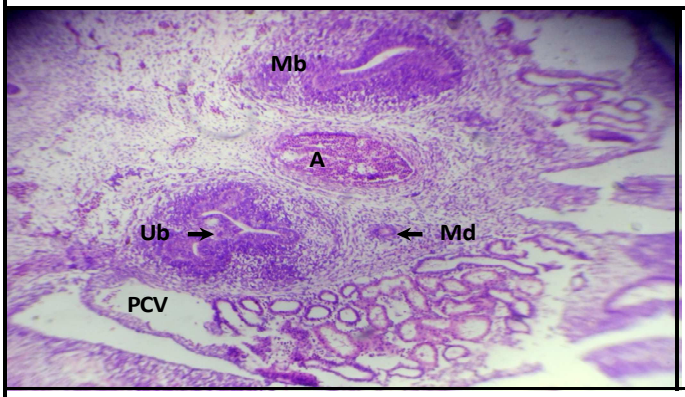
Fig.27 Photomicrograph of 39.5 day old sheep embryo showing ureteric bud.

H&E X400

A- Aorta
Ub-Ureteric bud
Mb-Metanephric blastema
Md- Mesonephric duct
PCV-Posterior Cardinal vein

Fig.28 Photomicrograph of 24 day old sheep embryo showing mesonephros and ureteric bud

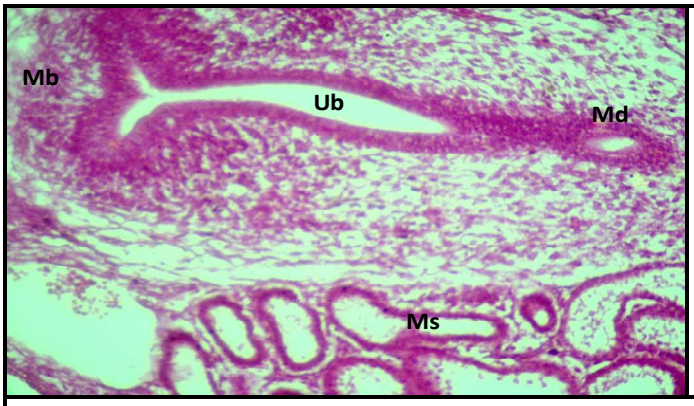
H&E X100



A- Aorta
Ub-Ureteric bud branchinng
Mb-Metanephric blastema
Md- Mesonephric duct
PCV-Posterior cardinal vein

Fig.29 Photomicrograph of 25 day old sheep embryo showing ureteric branching.

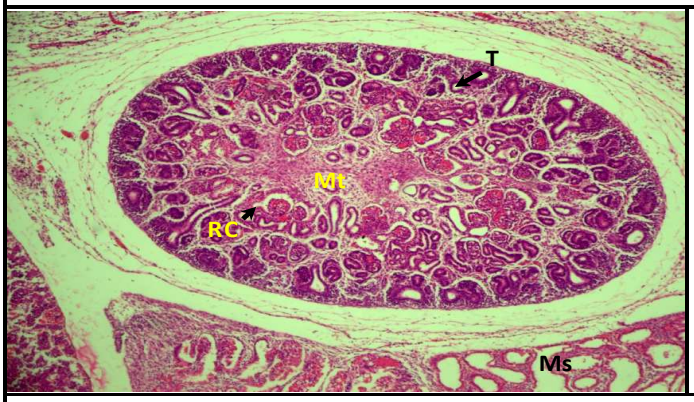
H&E X100



Ub-Ureteric bud
Mb-Metanephric blastema
Ms- Mesonephros
Md- Mesonephric duct

Fig. 30 Photomicrograph of 27 day old sheep embryo showing ureteric bud.

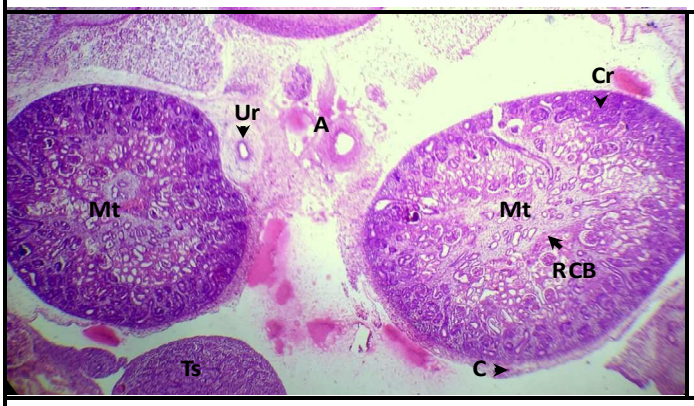
H&E X400



Mt-Metanephros
T-Developing tubule
RC-Developing Renal corpuscle
Ms- Mesonephros

Fig. 31 Photomicrograph of 37.5 day old sheep embryo showing metanephros.

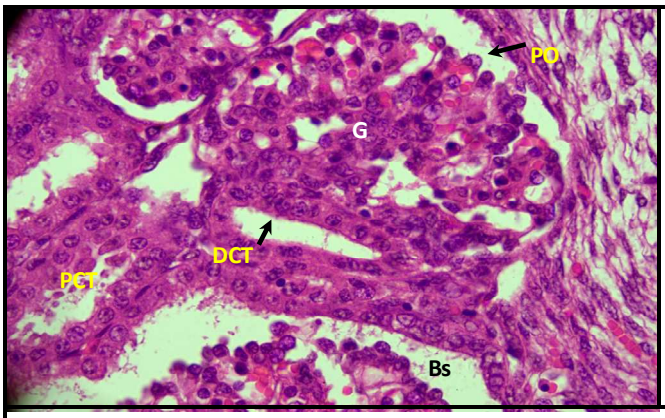
H&E X100



Mt-Metanephros
RCB-Renal columns of bertini
A-Aorta
C-Capsule
Cr-Cortex
Ts-Testes
Ur-Ureter

Fig. 32 Photomicrograph of 44 day old sheep embryo showing metanephros.

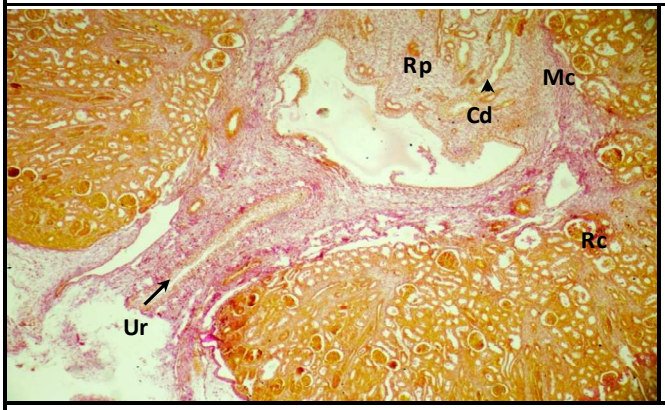
H&E X400



PO- Podocytes
G-Glomerulus
DCT-Distal convoluted tubules
PCT-Proximal convoluted tubules
Bs- Bowman's space

Fig.33 Photomicrograph of 44 day old embryo showing renal corpuscle.

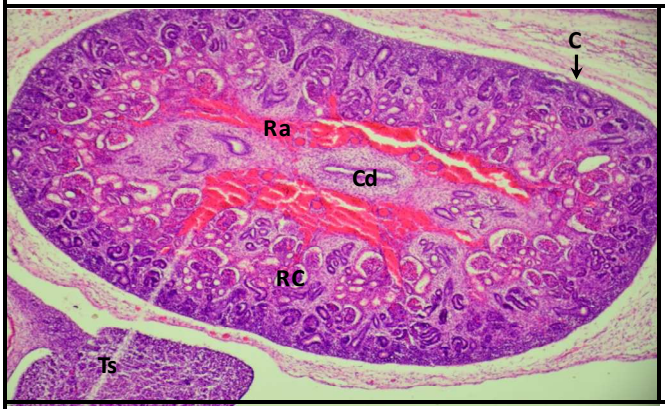
H&E X1000



Mc-Minor calyx
Rp-Renal papilla
Cd- Collecting ducts
Ur-Ureter
Rc-Renal corpuscel

Fig.34 Photomicrograph of 50 day old sheep embryo showing metanephros.

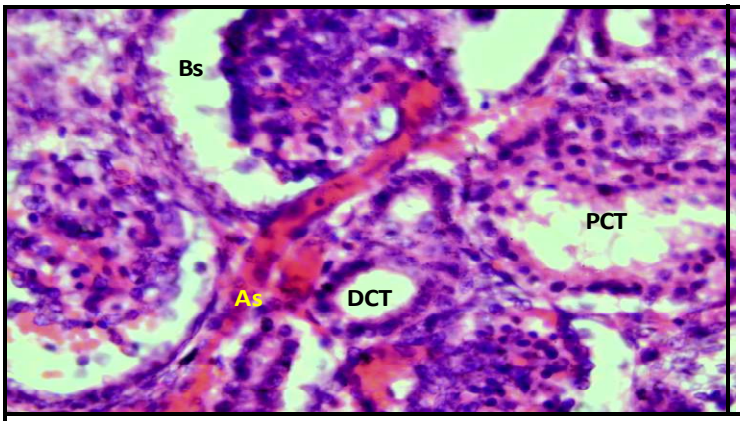
Verhoeff's X400



Cd- Collecting ducts
Ra-Renal artery supply
RC-Renal corpuscle
Ts-Testes
C-Capsule

Fig.35 Photomicrograph of 50 day old sheep embryo showing metanephros.

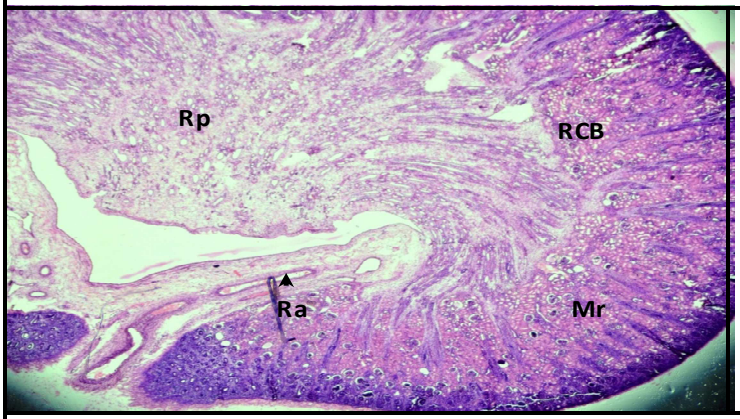
H&E X400



As- Arterial supply
PCT-Proximal convoluted tubule
DCT-Distal convoluted tubules
G-Glomerulus
Bs-Bowman's space

Fig.36 Photomicrograph of 50 day old sheep embryo showing renal corpuscle.

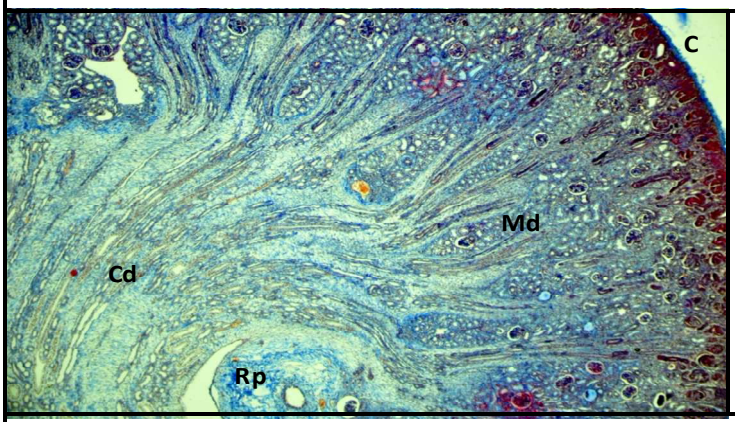
H&E X1000



Rp-Renal papilla
Ra-Renal artery
RCB-Renal columns of bertini
Mr-Meduallary rays

Fig. 37 Photomicrograph of 71 day old sheep foetus showing metanephros.

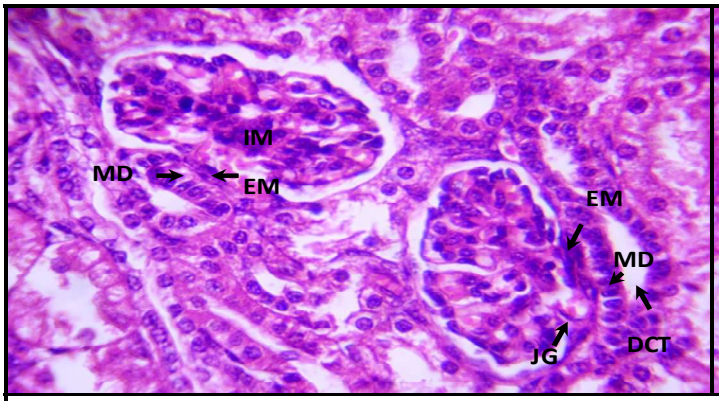
H&E X100



C-capsule
Cd-collecting duct
Md-Medullary rays
Rp-Renal papilla

Fig. 38 Photomicrograph of 78 day old sheep foetus showing metanephros.

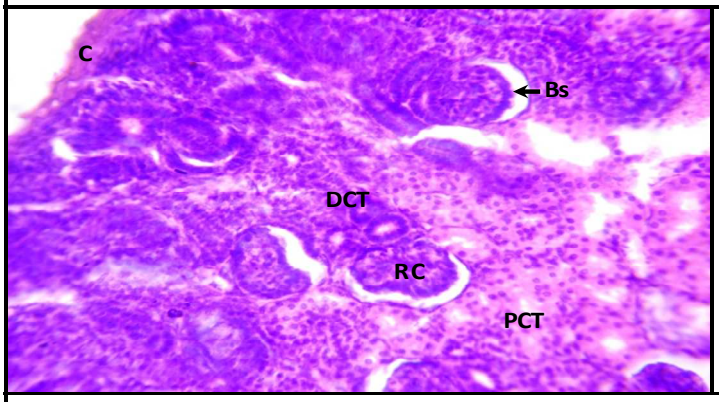
Masson'sTrichromeX400



EM-Extra mesengial cells
IM-Intra mesengial cells
MD-Macula densa cells
JG-Juxta Glomerular cells

Fig.39 Photomicrograph of 96 days sheep foetus showing macula densa cells in nephrons.

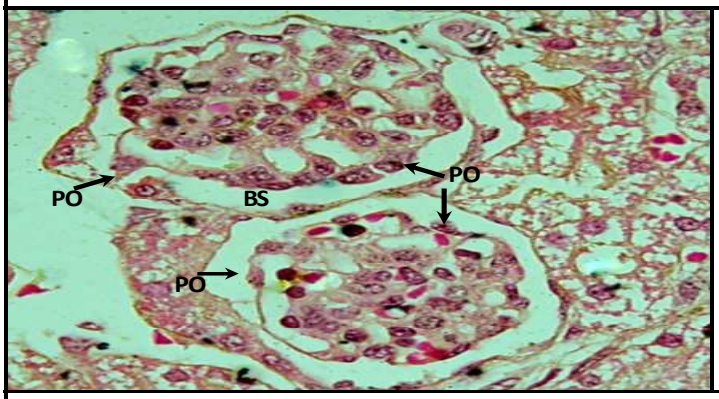
H&E X1000



C-capsule
DCT-Distal convoluted tubules
PCT-Proximal convoluted tubules
Bs- Bowman's space
RC-Renal corpuscle

Fig .40 Photomicrograph of 101 day old sheep foetus showing cortical nephrons.

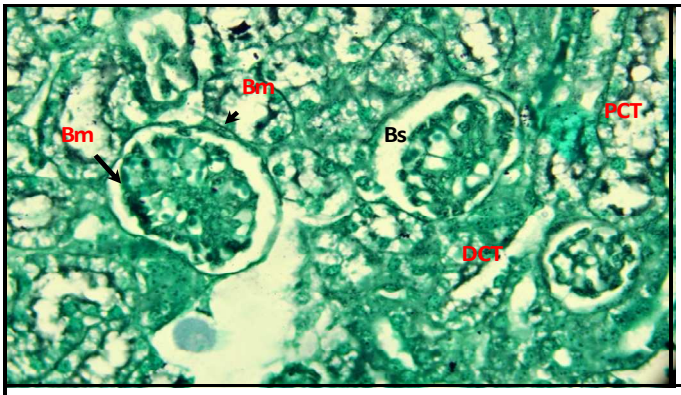
H&E X400



PO-Podocytes
Bs- Bowman's space
RC-Renal corpuscle

Fig .41 Photomicrograph of 107 day old sheep foetus showing podocytes.

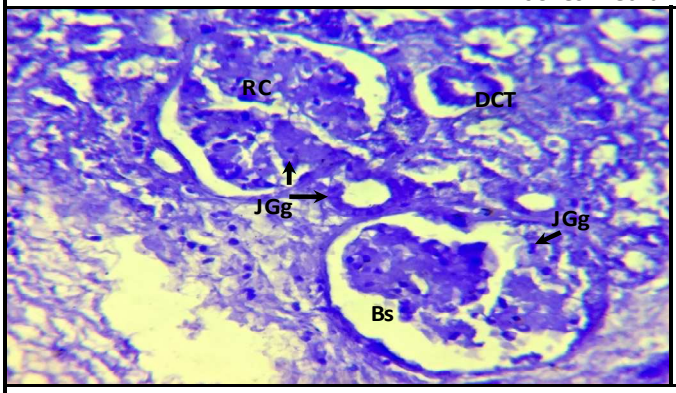
Jones Methanamine Silver X1000



Bm-Basement membranes
DCT-Distal convoluted tubules
PCT-Proximal convoluted tubules
Bs- Bowman's space

Fig. 42 Photomicrograph of 115 days sheep foetus showing basement membrane.

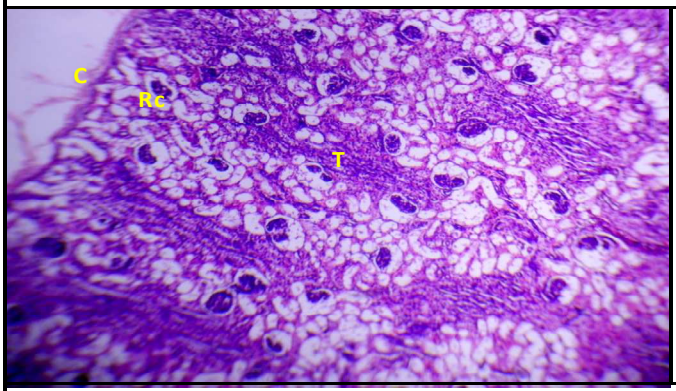
Jones Methanamine Silver X1000



JGg-Juxta Glomerular granules
DCT-Distal convoluted tubules
PCT-Proximal convoluted tubules
Bs- Bowman's space

Fig. 43 Photomicrograph of 120 day old foetus showing juxta glomerular granules.

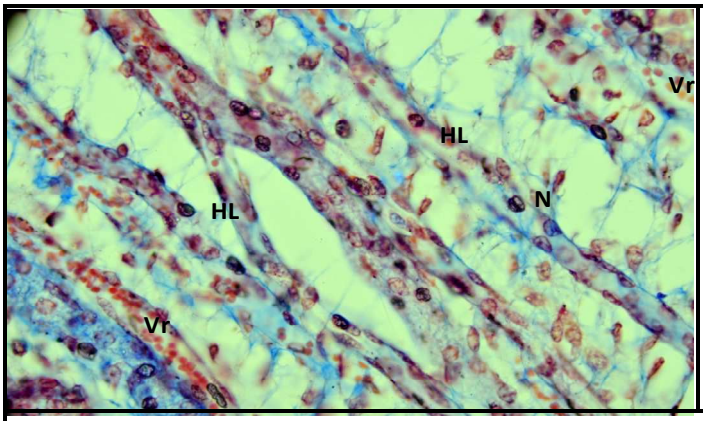
Harada X1000



C-Capsule
T-Tubules
Rc-Renal Corpuscle

Fig. 44 Photomicrograph of 144 day old sheep foetus showing cortical renal corpuscles.

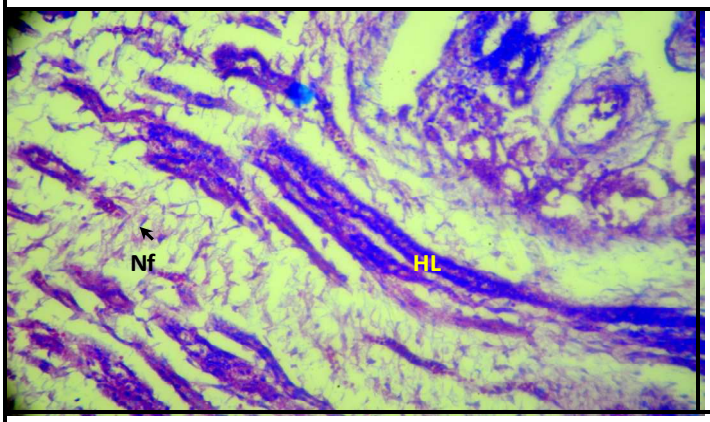
H&E X100



HL-Henle limbs
Vr- Vasa recta
N-Nuclei

Fig. 45 Photomicrograph of 120 day old sheep foetus showing Henle limbs

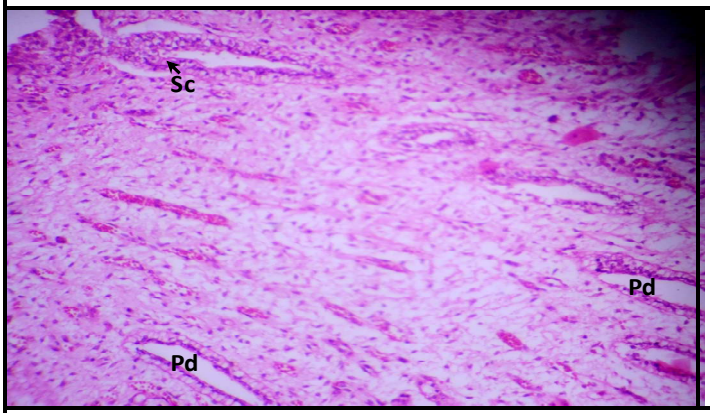
Masson's trichromeX1000



Nf-Nerve fibers
HL- Henle limbs

Fig.46 Photomicrograph of 115 day old sheep foetus showing nerve fibers.

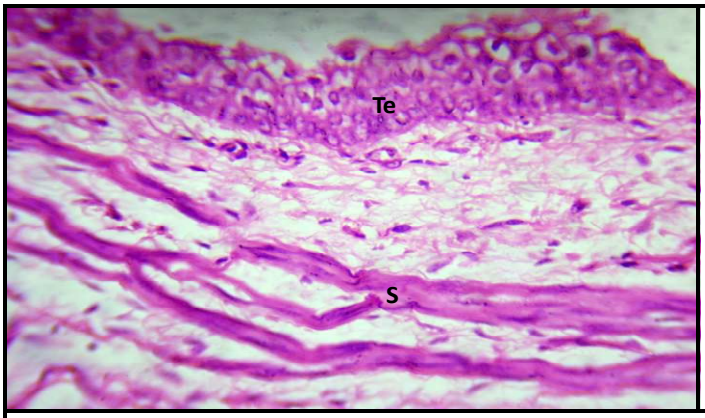
Luxol Fast blue X1000



Sc-Stratified cuboidal epithelium
Pd-Papillary ducts

Fig.47 Photomicrograph of 115 day old sheep foetus showing papillary ducts.

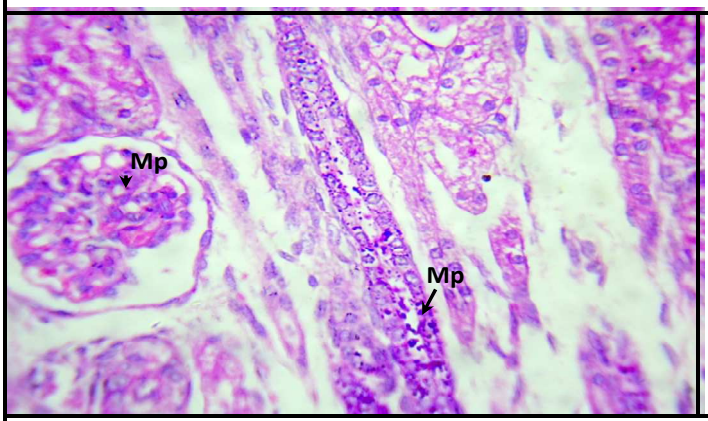
H&E X400



Te-Transistional epitheium
S-Smooth muscle fiber

48. Photomicrograph of 115 day old foetus showing minor calyx.

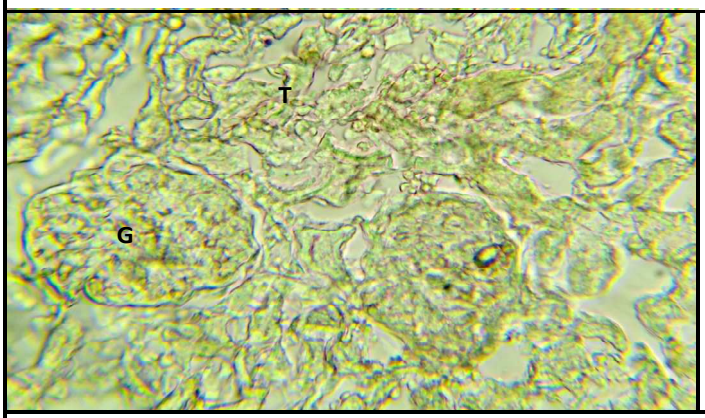
H&E X400



Mp-Muco polysaccharides

49. Photomicrograph of 120 day old sheep foetus showing muco polysaccharides.

PAS X400



G- Glomerulus
T- Tubules

50. Photomicrograph of 144 day old foetus showing negative reaction in metanephros.

Alkaline Phosphatase X1000

CHAPTER V

5.0 DISCUSSION

5.1 Morphogenesis of kidney

5.1.1 Location

In the present study, mesonephros in group I embryos at 20 days (0.7 cm CRL) was located lateral to the testis and occupied larger part of the coelomic cavity. Cranially, it was continued by pronephros and caudally, it opened with the mesonephric duct into the urogenital sinus. At 39.5 days (3.2 cm CRL) of gestation, mesonephros was located between metanephric kidney cranio-medially and testes caudo-laterally and by 41 days (4 cm CRL), it was located at the base of the metanephric kidneys.

The mesonephros was further extended towards the caudal end in 42.5 days embryos (4.5 cm CRL) and placed more caudally at 48 days (6.5 cm CRL). Medially, mesonephros was related to testes at 50 days which was slowly modified into ductus differens in male at 58 days (10 cm CRL) and uterine tubes in female at 60 days (12 cm CRL). Due to decrease in size from 41 days than the earlier age, the mesonephros and testes were pushed towards posterior end of metanephric kidney at 50 days in male foetus (10 cm CRL) and at 60 days (12 cm CRL) in female foetus. The anterior end of metanephros was attached to the posterior extremity of the adrenal gland at 41 days of gestation. Similar observations were made by Bello *et al.* (2012) and Morovatisharifabad and Salehi. (2015) in 60-120 days camel foetus.

5.1.2 Colour and Shape

At 39 days of gestation (3.2 cm CRL), the mesonephros was cream in colour and appeared as elongated and oval shaped structure. Its cranial border was rounded and caudal

border was tapered giving the appearance of elongated bulged cord like structure at 41days (4 cm CRL).

The metanphric kidneys of all age groups varied in colour from light to dark brown. Similar observations were made by Bello *et al.* (2012) and Morovatisharifabad and Salehi (2015) in camel embryos by *in vivo* studies. The shape of metanephric kidneys was irregularly ovoid upto 39.5 days (3.2 cm CRL), smooth, covered with a thin fibro-muscular capsule and changed to bean shape with rounded extremities at 41 days of gestation (4 cm CRL). These are in agreement with the findings of Bello *et al.* (2012) and Morovatisharifabad and Salehi (2015) in 90 days camel foetus and Jafar (2014) in 60-65 days buffalo foetus. However, Malik and Vais (1998) reported that the shape of kidneys was irregularly ovoid upto 10 cm CRL and changed to bean shape with rounded extremities in 20 cm and above CRL in goat foetuses.

5.1.3 Morphometry

Morphometric analysis of kidney revealed that there was no significant difference within the groups between left and right kidneys and there was no significant difference in group I and Group II in terms of weight and width, but there was significant difference in weight and width of both kidneys in group III. There was a significant difference in length in all age groups. In contrary, Malik and Vais, (1998) described that the right kidney was heavier (11.30 g) than the left (10.66 g) in all age group foetuses and all the parameters of right and left kidneys varied in different age groups. The length, thickness and cross sectional area of right kidney were greater those of left kidney except width, which was more in left kidney up to 20 cm CRL goat foetuses. However, Gopinath *et al. (*1997) observed unnoticeable difference in respective measurements within the foetuses of same group at all ages (0-150 days) although length, width, thickness and weight were found to increase with concomitant increase in the body weight and crown-rump length (CRL) of the

foetus as age advanced. Further, Salehi and Morovathi (2012) recorded variation in growth between the left and right kidneys of camel in terms of length and width at 45 days and 60 days of gestation. Significant variation in thickness was recorded between the right and left kidneys at 45 days and 120 days of gestation.

5.2 Histogenesis of Kidney

5.2.1 Pronephros

The pronephros in 20 days (0.7 cm CRL) old sheep embryo was placed on either side of the dorsal aorta at the level of body somites and ventral to the intermediate cell mass towards the dorsal median line. It was surrounded by undifferentiated mesenchymal cell mass called the nephric ridge. Davies (1951) reported that the intermediate cell mass was present at 7^{th} segment from its anterior to caudal end and appeared as a wellmarked dorsal bulge reffered to as nephric ridge. Pronephric vesicles were surrounded by a layer of mesenchymal cells and fibers. At 24 days (1.1 cm CRL) of gestation, the pronephros was surrounded by a thin layer of connective tissue capsule. It was lined by squamous to cuboidal cells but the posterior pole of pronephros that respresented the future opening of pronephric duct was lined by stratified cuboidal cells as mentioned by Rao and Jagapthiramayya (2013).

The degeneration of pronephros was first observed at cranial pole in 27 days (1.7 cm CRL) embryo. The mesenchymal covering around the pronephros disappeared by leaving few tubules. The rest of the pronephros was filled with differentiating mesenchymal cells and completed the process of degeneration by 31 days (2 cm CRL) of gestation in sheep embryos. In contrary, Davies (1951) recorded that the pronephros persisted until late stages of embryonic life in sheep foetus.

5.2.1.1 Pronephric glomeruli

Intense angioblastic activity was observed on either sides of the dorsal aorta in 20 day old embryos (7 mm CRL). The pronephros consisted of only one giant glomerulus of 225 ± 1.3 µm in size, which was placed medially and varied from middle to ventral in its location. The whole of the glomerulus was surrounded by very thin layer of cuboidal cells with connective tissue fibers. These were in accordance with Davies (1951) at 14 somite stage in sheep embryos. However, Rao and Jagapathi Ramayya (2013) denoted them as external glomeruli in mammals.

At 24 days of gestation (1.1 cm CRL), the giant glomerulus was surrounded by a distinct capsular space only at certain places. The pronephric glomerulus was surrounded by a single layer of squamous to cuboidal cells at posterior pole. These cells appeared as tall cuboidal cells indicating the tubular continuation. However, Devies (1951) identified that the glomerular complex was almost fully developed and probably functional at 29 somite stage in sheep. In the present study, degenerative changes were noticed in glomerular network due to which vacuolation was noticed between the tuft of glomerulus at this age. Glomerulus was not developed in association with the cranially situated nephric tubules. It ultimately disappeared and complete degeneration of glomerulus was noticed as indistinct glomerulus in the pronephros by about 27 days (1.7 cm CRL). No reviews are available in this regard.

5.2.1.2 Pronephric tubules

In 20 days old sheep embryo (0.7 cm CRL), many tubules were present in the pronephros. Some of them were fully formed while others were incomplete open type. Periphery of the pronephros consisted of irregular tubules that varied in size. The tubules present at the dorsal end of the vesicles were elongated and irregular in shape and traversed towards the caudal part of the vesicle. Tubules present close to the glomerulus appeared as incompletely oriented cluster of angioblast cells. The caudal ends were connected to

coelomic epithelium by cellular strands like stalks that probably represented the rudimentary peritoneal funnels on either side. Davies (1951) observed similar changes at 23 somite stage in sheep. About forty-five tubules in various ages of development were reported with the most rudimentary tubule lying caudally. At 29 somite stage, increase in length and an early differentiation of the cranial tubules into secretory and a collecting segment was elucidated.

At 24 days of gestation (1.1 cm CRL), the mesenchymal differentiation progressed. The number of tubules were 6 to 7 and restricted to caudal end of pronephros. They were lined by cuboidal cells with discontinuous brush borders. The lumen of the tubules was occupied by eosinophilic material. The tubules increased in size upto this foetal age and were located postero-dorsal to the glomerulus. The degeneration of the tubules was observed from cranial end to the periphery of the pronephros at 25 to 27 days of gestation (1.5 cm CRL to 1.7 cm CRL). The number of tubules was restricted to 3 to 6 and were lined by large/tall cuboidal cells with prominent centrally placed nucleus surrounded by eosinophilic cytoplasm. Some tubules had brush borders with little narrow lumen whereas the other tubules were without brush border. The size of the tubules started decreasing at 25 days than the earlier gestation periods. There was increase in the concentration of cellular strands at the caudal end and length of the stalk increased thus indicating the union of pronephros with primary excretory duct, which appeared as solid rod of cells in the coelomic cavity. At 40 days of gestation (3.7 cm CRL), a stalk of degenerated pronephros was attached to the cranial pole of the mesonephros at the level of 12^{th} to13^{th} vertebrae. No reviews were available for these ages.

5.2.2 Mesonephros

The mesonephros was established on the caudal portion of pronephric duct, immediately behind the pronephric tubules. In 20 day (0.7 CRL) old sheep embryos, the mesonephric vesicle was globular in shape and occupied the dorsal part of coelomic cavity from below the level of somites and cranially it was adjoined to the pronephros by primary excretory duct. These results were in accordance with the findings of Gopinath and Singh (1998) in goat embryo of 6 mm CRL (15 days). The mesonephros in the present study was present on the ventro-lateral aspect on left side, nearly ventral to the aorta on right side and was related to the archenteron on the ventro-medial aspect. It was surrounded by many layers of mesenchymal cells, which were interspersed by connective tissue fibers.

In 20 days sheep embryo, mesonephros was densely packed with tubules, migrating angioblasts and differentiating glomerulus. At 24 days (1.1 cm CRL), it was club shaped and connected medially to the gonads by a stalk like structure. It was surrounded by a thin capsule of 14 ± 1.2 μm and made of connective tissue fibers. The mesonephric parenchyma was differentiated into glomeruli and tubules with scanty differentiating mesenchymal cells at the posterior border of mesonephros. Angiogenic mesenchyme cells were traversing in between the tubules and periphery of mesonphros contained large blood capillaries. These changes are in agreement with Suman *et al.* (2013) in buffalo foeti from 2.5 cm CRL onwards.

At 25 to 27 days (1.5 cm CRL) of gestation, the parenchyma was little reduced than the earlier age, thickness of capsule increased to 17 ± 1.4 μm without elastic fibers and the blood supply from the aorta was established predominantly to both the mesonephric glomeruli. Posterior cardinal vein extended anteriorly over the tubules and ventrally mesonephros consisted of Wolffian duct. Reviews pertaining to these changes are lacking.

Between 29 to 31 days of gestation (2 cm CRL), mesonephros was related to the 7-8th somite cranially and extended upto the 16th somite caudally. The shape of the

mesonephros was changed from globular to elongated/shuttle and covered with a thick capsule of 32 ± 1.5µm in 37 to 39.5 days (3.5 cm CRL) embryo when compared to the earlier age. The parenchyma was densely packed with many glomeruli and tubules.

Sheep embryo of 39.5 days (3.5 cm CRL) showed mesonephros that prolonged to 18th somite in the coelomic cavity. The degenerative process was initiated in the anterior pole at this age. Typical elongated shuttle shaped mesonephros was present medial to the metanephric kidney with medial concave and lateral convex surfaces. At 49 days (7 cm CRL) of gestation, the mesonephros was surrounded by a thick capsule which consisted of connective tissue fibers. At the anterior pole, the degenerative process of the parenchyma was progressively increased than the earlier ages. The concavity towards the medial border was increased and accommodated testes into it. However, Gopinath and Singh (1998) described the structural distortion of mesonephric tubules that marked the onset of degenerative process in cranial zone of the organ at 18 mm CRL (30th day). The degenerative changes advanced from cranio-caudal to middle and adjoining one-third of caudal zone of mesonephros at 22 mm CRL (34 days) in goat foetus. Suman *et al.* (2013) reported that the cranial part of mesonehoros degenerated earlier than caudal part in 3.5 cm CRL buffalo foeti. They observed that glomeruli lost their walls and large masses of glomeruli were noticed in cranial mesonephros at 5.7 cm CRL. More prominent degenerative changes were evident at 7.2 cm and 10.3 cm CRL and advanced stages of degeneration were noticed in 10.3 cm CRL buffalo foeti.

At 57 days (10 cm CRL), the distance between testes and the mesonephros was reduced than earlier age. Mesonephros was closely adherent to the testes and its parenchyma was replaced with connective tissue. It was covered by thick capsule, lined by squamous cells and later become ductus deferens of the testes at 74 days. Conversely, Chandra (1964) and Gopinath and Singh (1998) mentioned the complete degeneration of

mesonephros at 84 and 95 cm CRL in goat foetuses. Further, Rao and Jagapathiramayya (2013) reported that the mesonephros regressed in horse around 65th day of gestation, in cattle at 58 days, pigs around 50 days and in dog at 36 days.

5.2.2.1 Mesonephric glomeruli

At 20 days of gestation (0.7 cm CRL), the mesonephros showed giant glomeruli, which filled nearly the whole of the Bowman's cup by leaving a small narrow space. Bremer (1916) observed similar findings in cattle and deer embryos. Giant glomeruli in sheep embryos were formed by the fusion of the most of the cranial 15-20 mesonephric corpuscles as per Davies and Davies (1950) and Davies (1951). The capsule was lined by squamous to cuboidal cells. Larger groups of mesenchymal cells and angioblasts comparative of giant glomeruli were seen in ventro-medial region. These results are in agreement with Rao and JagapathiRamayya (2013) in domestic animals. However, Fraser (1920) reported that the glomeruli first appeared at 35-36 somites caudal to the 12th segment in cat.

No noticeable difference was observed in the mesonephros of sheep embryos between 24 to 25 days (1.1 to 1.5 mm CRL) when compared to the preceding stage. Both the cranial and caudal parts of mesonephros were differentiated and capsular space was clearly noticed. The thickness of the capsular space was 14 ± 1.2 µm. The glomerular mass with tuft of capillaries filled the entire cavity of the Bowman's capsule. The size of the glomerulus was 210 ± 2.4µm. Gopinath and Singh (1998) made similar observations at 24 days (14 mm CRL) and 30 days in goat fetuses. Visceral and parietal layers around glomerulus were demarcated at this age. They were lined by simple squamous epithelium. In the visceral layer, very few cells between the squamous cells were modified and presented with large vesiculated nucleus and formed the future podocytes. Collagen fibers were interspersed between the tufts of glomeruli. Anterior pole of the glomeruli showed

reticulocytes on one side and developing tubules on the other side. At 24 to 25 days, individual transverse branches from the primitive aorta passed along the mesonephros to the glomerulus. The glomerular columns in the middle zone converged dorso-medially to join the mesonephric tubules at the dorsal end and the number of glomeruli increased in cranio-caudal zone in sagittal view. In 35.5 days sheep embryo (3 cm CRL), the glomeruli were centrally placed in the mesonephros, oriented in a linear fashion and extended from the cranio-medial to the caudal pole of mesonephros. They appeared as many lobules with basal and apical connections at this age. These observations were in accordance with the findings of Davies and Davies (1950) in sheep where the nephrons reached their maximal number of almost 70. The manifestation of macula densa of DCT, Juxta glomerular cells of afferent arteriole and mesangial cells of smooth muscle at vascular pole of Bowman's capsule indicated the appearance of the juxta glomerular apparatus in 27 days fetuses. However, Davies and Davies (1950) reported the glomerular maturity at 21st day in sheep and 26th day in cattle embryos.

The degenerative changes were advanced in cranio-caudal border to middle and adjoining 1/3rd part of the caudal zone of mesonephros in embryos of 39.5 days (3.5 cm CRL) sheep foetuses. The mesonephric glomeruli at this location lost their epithelial organization and the size was reduced. The relative size of the glomeruli decreased over that of the preceding group. At 49 days (7 cm CRL) of gestation in sheep, the glomerulus was shrunken, the parietal layer of glomerulus was thickened with increased capsular space. At 50 days (7.5 cm CRL), the relative number of glomerulii was further reduced. The parietal cells of Bowman's capsule at the urinary pole were slowly transformed into cuboidal cells with increased capsular space. At 57 days (10 cm CRL), the fragmented glomeruli with indistinct visceral layer and increased Bowman's space could be observed in whole of the mesonephros. These results are in accordance with the findings of Davies and

Davies (1950) in sheep. In contrary, Bremer (1916) reported no regression of mesonephros in 49th day cattle embryo.

5.2.2.2 Mesonephric tubules

Sheep embryos of 20 days (0.7 cm CRL) showed many of irregular tubules, which were histologically differentiated through the condensation of mesenchymal cells. Some of them were epithelialized and formed vesicles with narrow and wide lumen in the parenchyma of mesonephros. Some tubules were without any evidence of substance in their wide lumen representing the DCT. These tubules were in the middle surrounding the glomeruli. Some of the tubules were closely related to the anterior and posterior poles of glomeruli. The cranial and caudal zones of mesonephros possessed clusters of eosinophilic angiogenic mesenchymal cells, which were progressively differentiated into PCT. The periphery of the mesonephric parenchyma consisted of well-defined mesonephric vesicles, many of which showed ventral elongation as progenitors of mesonephric tubules. These were usually straight, lined with simple cuboidal epithelium of uniform height, had wide lumen and were without brush borders. Similar findings were observed by Suman *et al.* (2013) in 3 cm CRL buffalo foetus.

In 24 days embryo (1.1 cm CRL), the mesonephric tubules were grown in length than the earlier age and took an elongated/comma shaped curves. Tubules with thin wall and wide lumen appeared at the periphery of mesonephros and were lined by cuboidal cells. Most of the tubules established continuity with the existing mesonephric duct. The angiogenic cells at places reorganized themselves as capillaries. The relative number and size of the mesonephric tubules were increased than in previous gestation period. Some tubules had wide lumen and were lined with cuboidal cells. The nuclei were placed basally and cytoplasm showed foamy appearance with eosin. Most of the tubules in the parenchyma showed histological differentiation into the proximal and distal segments.

Nucleated blood cells were noticed between the tubules in the parenchyma. In contrast, Gopinath and Singh (1998) stated in goat foetus at 6 mm CRL (15 days).

The proximal and distal segments of mesonephric tubules were sharply defined in sheep embryos of 25 days (1.5 cm CRL). The mesonephric duct was located at the periphery in the postero-lateral zone and was lined by cuboidal cells. The middle and caudal zones revealed no organizational changes over the preceding stage. Though the present findings were in accordance with the observations of Suman *et al.* (2013) in 3 cm CRL buffalo foetus, the tubules occupied the greater area of caudal part of mesonephros. At 27 days (1.7 cm CRL), the mesonephric duct was present at the caudal aspect of the gland.

At 31 days (2 cm CRL), the proximal and distal segments of mesonephric tubules were easily identifiable based on the differences in height and staining affinities of their epithelial cells. The proximal tubules possessed narrow lumen with brush borders. Thin walled irregular sized collecting tubules were noticed at the periphery of the gland. At 35.5 days (3 cm CRL), $3/4^{th}$ of parenchyma was occupied by densely packed tubules, which were irregular in shape and presented towards the periphery/lateral aspect of mesonephros. Some of them were lined by cuboidal cells with narrow lumen where as others were lined by squamous to cuboidal cells with wide lumen. Similar observations were made by Suman *et al.* (2013) in 3 cm CRL buffalo foetus. At this age, there were striking similarities in structure between the mesonephros and metanephros but there was no demarcation between cortex and medulla and Henle's loop was absent. These observations were in concord with Rao and Jagapthi Ramayya (2013) in domestic animals.

The structural distortion of mesonephric tubules marked the onset of degenerative process and atrophied in the cranial zone of the mesonephros in 39.5 days sheep embryo (3.5 cm CRL). The cranial tubules lost their epithelial cell lining and stained more intensely. Though the present findings were in agreement with the observations of Davies

(1951), the degeneration was identified at 26th day in cattle and 27th day in sheep. The tubules in the middle zone were reduced in size. Some tubules lost their epithelium and the caudal tubules were still developing. There was disappearance of epithelial organization in the tubules at 44 days (5 cm CRL), but the concentration of the tubules was decreased by 46.5 days (6 cm CRL) and the size of the capsule was increased.

Complete cellular disintegration of tubules was noticed in 1/3rd of the cranial mesonephros and 2/3rd tubules showed small vacuolations in the tubular cells at 49 days (7 cm CRL). At 57 to 59 days of gestation (10 cm to 12 cm CRL) 3 to 4 mesonephric tubules persisted at the cranial end as aberrant ductules. The succeeding 7-8 tubules were little larger than the preceeding. At 74 days (16 cm CRL), 12-13 tubules in cranial part towards testes were round to elongated and were lined by cuboidal cells with narrow lumen. They developed into efferent ductules of testes following which some of the tubules were rounded with little wider lumen than preceding ones indicating future head of the epididymis.

5.2.3 Metanephric kidney

5.2.3.1 Ureteric bud

The ureteric bud formation by the evagination of the caudal part of the mesonephric duct in sheep embryo was noticed first at 20 days of gestation (0.7 cm CRL). The entire renal duct system developed from this ureteric bud. It possessed a very narrow lumen, which represented the future lumen of ureter. However, Patten (1949) identified at 5 mm CRL in pig and Gopinath and Singh (1998b) at 8 mm CRL (17 days) in goat embryo.

At 24 days (1.1 cm CRL) of gestation, the ureteric bud was placed on either side of the dorsal aorta and at its caudal end had an attachment to the mesonephric duct that opened into the urogenital sinus. Angiogenic mesenchyme migrated into the metanephric blastema slightly later to produce the glomeruli and vasa recta. Ureteric bud elongated cranio-

caudally and the cranial end was ampullated. Similar findings were made by Gopinath and Singh (1998). Further, they described that the elongated ampullated duct differentiated into 2 primary branches as intra renal duct system in 14 mm CRL (24 days) goat embryos.

Between 25 to 30 days of gestation (2 cm CRL), the ureteric bud underwent a series of bifurcations within surrounding metanephric mesenchyme and formed varied number of smaller ureteric ducts. At the same time, the metanephric mesenchyme condensed around the dividing ducts and formed S-shaped clusters, which transformed into epithelia and fused with the ureteric ducts at their distal ends. The branching was completed and the metanephric blastema was fully transformed to rounded cap like structures with poorly developed metanephric parenchyma that enclosed primitive tubules and vesicles in it. The relative density of cells mass increased gradually as age advanced. Cuboidal cells with eosinophilic cytoplasm and basophilic nuclei lined the primitive tubules.

5.2.3.2 Metaneprhric blastema

The ureteric bud was surrounded by a mass of mesenchymal cells. The bud elongated cranio-caudally, the cranial end was ampullated and capped by nephrogenic/metanephric blastemal cells in 24 days sheep embryos (1.1 cm CRL) as reported by Gopinath and Singh (1998) in 12 mm CRL goat embryos. However, Chandra (1964) described these changes in 13-19 mm CRL goat embryos. Conversely, Tiedeman (1976) described that kidneys started developing by 27 to 30 days of gestation in sheep; Hoster *et al.* (1999) during 21 days to 28 days of gestation in pig; Gaeth *et al.* (1999) at 139 to 166 days in elephant embryo; Suman *et al.* (2008) at 45 days in buffalo foetii and Hyttel *et al.* (2010) at 25 days in cow embryo.

The mesenchymal clusters were converted into small groups of epithelial cells that underwent complex morphogenic changes between 25 to 29 days in sheep embryos. Each epithelial group was first elongated to form comma-shaped body followed by an S-shaped

body. It continued to elongate and subsequently fused with a branch of the ureteric duct at its distal end and expanded as a dilated sac at its proximal end. The blood vessels invaded the proximal ends of the S-shaped tubule to form vascularized glomeruli and ureter emerged out from the hilus of the kidney. The mesenchymal cells bordering the whole of the nephrogenic mass condensed at periphery as primordial capsule of the developing metanephric kidney in 29 day old embryos (1.9 cm CRL). In para-cortical region, 2 to 3 glomeruli were differentiated with capsular space in 36 days embryo (2.5 cm CRL). These observations are in agreement with the findings of Gopinath and Singh (1998b) in 18 mm CRL (30 days) goat embryos and Jafar (2014) during 60 to 65 days of gestation in bovine.

5.2.3.3 Renal corpuscle

At 37.5 days (3 cm CRL), metanephric kidney was surrounded by undifferentiated mesenchymal cell mass that invaded from the periphery to the centre between the tubules and it formed the future lobules and pyramids of the kidney. There was no demarcation between cortex and medulla at this age. The number of glomerular masses increased when compared to earlier ages and were suspended between the tubules and centre of the gland. Some renal corpuscles, showed glomerulus with clear Bowman's capsule. The nephrogenic mass surrounding glomerulus was differentiated as parietal capsular cells and inner visceral cells surrounding capillary network. Metanephric kidney was divided into dark coloured cortex and lighter coloured medulla at 44 days (5 cm CRL) of gestation. The renal corpuscles were distributed between the para cortical zone and the medulla. The size of the renal corpuscle was 75 ± 1.6 µm. Few renal columns of Bertini were noticed at 44 days of gestation. Tuft of glomerular capillaries and Bowman's space were evident in each renal corpuscle. Parietal layer was lined with squamous cells and the visceral layer was closely adherent to the glomerular network as podocyte like cells. At the vascular pole of the glomerulus, distal convoluted tubules were identified but there was no evidence of Juxta

glomerular cells in this age group. Similarly, Jafar (2014) described the beginning of nephron formation during 60-65 days of gestation in bovines. The metanephric mesenchyme responded to the invading bud by proliferation and then condensation to form coronas of cells around each branch of the bud. Within the condensed mesenchyme, the cells converted into epithelial cells. However, Gopinath and Singh (1999) described that the nephrons of goat developed first in the juxta-medullary zone at 45 mm CRL (50 days) and subsequently in the intermediate and sub capsular zones of the cortex in embryos of 55 mm CRL (52 days) and 70 mm CRL (56 days) respectively. Bello *et al.* (2013) identified few developing glomeruli in the cortex of camel kidneys below 130 days of gestation in the first trimester.

The renal corpuscles increased in number and were distributed towards the periphery from juxta medullary regions of the parenchyma. Two to four corpuscles were obvious at the papilla that represented future apex of the pyramid. Internal lobulation was evident in the apical/cranial region of kidney from 50 days (7.5 cm CRL) of gestation onwards. The size of the capsular space increased to 80 ± 5.3 µm up to 57 days of gestation (10 cm CRL). Some cells between the endothelial cells were modified as mesengial cells. Vascular pole of renal corpuscles was more evident with blood supply into it. But there was no evidence of Juxta Glomerular complex cells. Renal corpuscles occupied the intermediate zone in 71 day old embryos. At 78 days, the capsular space reduced to 12 ± 2.1 µm when compared to the earlier ages. The PCT was lined by cuboidal cells with brush border in their lumen. At 96 days, Juxta glomerular apparatus cells were well developed in the intermediate nephrons.

In group III, the typical cortex and medulla were differentiated. The renal corpuscles were still under differentiating stages in the cortical region by 101 days. Capsular space was decreased than earlier groups. Juxta glomerular complex was present.

At 107 days, the cortex was clearly differentiated into cortical labrynth and medullary rays. Differentiation of renal cropuscles progressed when compared to the previous age. Capsular space was surrounded by parietal squamous cells and visceral podocytes. The basement membranes of the renal corpuscle were well demarcated by 115 days. Juxta glomerular cells exhibited cytoplasmic granules by 120 days and it indicated the functional kidney. At 144 days, histologically kidney was well differentiated as adult kidney and the cortical nephrons/renal corpuscles were distributed through out the cortex in sheep foetuses.

5.2.3.4 Metanephric tubules

The metanephric tubules were occupied through out the periphery and centre of the kidneys by 37.5 to 44 days. The tubules at the periphery were lined by cuboidal cells with narrow lumen representing the precursors of renal corpuscles but the tubules at the centre were lined with wide lumen. Demarcation between proximal and distal tubules was noticed from 44 days of gestation onwards. Majority of the mesenchymal mass in the parenchyma of the gland was differentiated into tubules in 50 days embryos. Between 50 to 60 days, the tubules present in the close proximity of the renal corpuscles were modified into proximal and distal tubules. Proximal tubules were lined by irregularly oriented flat to cuboidal cells with eosinophilic cytoplasm and distal tubules were lined with regularly arranged cuboidal cells and had wide lumen. Though they were closely adherent to corpuscles, the cells were not modified into macula densa cells and Henle's loop was not evident upto this age. But around the renal corpuscles, cluster of collecting tublues was prominently seen at this age. Loop of Henle first appeared at 83.5 days and were well established in Juxta medullary nephrons as descending and ascending limbs by 96 days. However, Gopinath and Singh (1999) studied that the proximal tubules arose in continuity of the Bowman's capsule from lower limbs in the juxta-medullary zone at 45 mm CRL (50 days). The distal convoluted tubules had their origin from the upper limbs of the primordial vesicles. Considerable

coiling and epithelial specialization was observed at 70 mm CRL (56 days). Henle's loop and distal convoluted tubules had their origin from the middle and upper limbs of the primordial vesicles in tubules at this age. The proximal convoluted tubules and distal convoluted tubules appeared better developed and more coiled in goat embryos of 120 mm CRL (74 days). The proximal convoluted tubules of goat foetii formed bulk of the cortex by 250 mm CRL (112 days) and segments in Henle's loop was observed at 118 days of gestation. Conversely, Suman *et al*. (2008) reported that in 3.0 cm CRL buffalo foeti, the peripheral parts of these tubules were dilated and showed dichotomous bifurcation. The branches of all the tubules were lined by multilayered epithelium with elongated nuclei. No clear-cut differentiation of renal cortex and medulla was observed at this stage, but the formation of S-shaped vesicles along with metanephric tubules could be seen. The PCT and DCT were first observed in 4.1 cm CRL buffalo foeti in the juxtamedullary area and PCT were lined by simple columnar epithelium in upto 5.7 cm foeti. The tubular segments of nephron were differentiated in the cortex as proximal and distal convoluted tubules in the buffalo foetii of 5.7 cm CRL onwards. PCT appeared in the intermediate and sub capsular areas of the metanephros in 10.3 cm CRL onwards. Formation of striations (future brush border) at the luminal end of the epithelial cells was first noticed at the age of 11.2 cm CRL and the juxtamedullary glomeruli were encapsulated. In 23 cm CRL foetii, PCT were lined by simple cuboidal to pyramidal type of cells along with luminal striations. The DCT were lined by simple cuboidal epithelium with relatively less eosinophilic cytoplasm and darkly stained nuclei. The DCT presented nuclear clustering regarded as the future macula densa of the juxtaglomerular apparatus. Similarly, Henle’s Loop was noticed at the foetal age of 7-8 cm CRL (60 to 65 days) in buffalo foetii. At 90-95 days of gestation, the proximal and distal convoluted tubules of buffalo foeti differentiated completely. Henle’s loop was undifferentiated relatively at this age. At 120 to 130 days, the proximal tubule was lined by

simple cuboidal cells that exhibited round light coloured large nucleus, with no brush border. The distal tubule cells were changed to tall cuboidal cells with rounded dark nucleus. The Henle's loop become visible and consisted of two parts. The first thin segment was lined by squamous cells and the second thick segment was similar to PCT (Jafar, 2014).

Renal pyramids were not apparent in 50 days sheep foetus except in the caudal aspect. At one place in the caudal region, medullary rays were extended from apical zone of the renal pyramid to the cortical area of the metanphric kidney. Increased quantity of collagen fibers was noticed at the pelvis than earlier ages but elastic fibers were not noticed. Renal sinuses were present at the hilus, ureters were entering into the hilus and prominent renal pelvis was noticed at 50 days. Major and minor calyces were apparent at 57 to 60 days of gestation and increased quantity of collagen was noticed at the pelvis, around the major and minor calyx and between and around the tubules in the cortical region. The parenchyma towards renal sinus was gradually modified into pyramid like structure whose apices were facing into developing minor calyces. In between these pyramids, the parenchyma was projected as columns of Bertini. Renal columns were more evident. The papillae opened into minor calyces, which coalesced together and opened into major calyces and then the papillary ducts opened into pelvis. These changes were more evident at 71 days than the earlier ages. Loop of Henle first appeared at 83.5 days, which were well established in Juxta medullary nephrons as descending and ascending limbs by 96 days. The renal columns of Bertni were well established by which renal pyramids were more obvious. Macula densa cells were identified in intermediate/cortical nephrons at this age. Nerve fibers were evident between the tubules in the medulla from 96 days onwards. Papillary ducts were lined by stratified cuboidal epithelium. Minor calyx was lined by

transitional epithelium like adult kidney and smooth muscles were also noticed in the wall of the calyx minor at 115 days of gestation that represented sphincter formation. Conversely, Jafar (2014) observed that the ureter was elongated and split into cranial and caudal evident portions (primary branches) that formed the future major calyces thus marking the beginning of duct system induction and up to ten funnel-shaped divisions were considered as minor calyces in 60 - 65 days buffalo foetus. At 90 to 95 days of gestation, the para-sagittal section of metanephros showed visible medullary rays composed of collecting tubule and collecting duct (duct of Bellini), which terminated with papilla. The collecting duct in cortex was lined by simple cuboidal epithelium and was gradually translated to columnar in medulla until it became pseudostratified epithelium at papillary duct. At 100 to 130 days of gestation, there was more evidence of collecting tubules and collecting duct. There was increase in the diameter of the individual collecting tubules and the down growth of the loops of Henle by 150 to 160 days.

5.2.3.5 Capsule and stromal elements

At 30 to 37 days (2 cm CRL), the mesenchymal cells were bordering the gland by forming a thin layer of capsule. A thin layer of capsule covered the whole metanephric kidney, which was eosinophilic with few connective tissue fibers (mainly collagen), fibroblasts and few reticular fibers towards the periphery of the capsule (3 cm CRL). At 45 days of gestation (5.5 cm CRL), the thickness of the capsule increased and was lined by single layer of squamous cells with few nerve fibers.

Similarly, Tanvi *et al.* (2015) found that mesenchymal cells of goat foetii condensed as a thin layer around the kidney (metanephros) as forerunners of the renal capsule at 45 days (5.3 cm CRL) and 46 days (6.3 cm CRL) of gestation. Thin fibro-muscular capsule covering the smooth surface of the kidneys was observed in camel fetuses at all ages (Bello

et al., 2013), buffalo foetus at 7-8 cm CRL (Jafar, 2014) and camel foetus at 90 days of gestation (Morovatishariabad and Salehi., 2015).

The collagen fibers were arranged in thick bundles with increased capsular thickness in 50 days (7.5 cm CRL) embryos. Elastic fibre content increased in the capsule of 57 days embryos (10 cm CRL). The thickness further increased with wavy bundles of collagen fibers interspersed with a thin layer of elastic fibers at 78 days of gestation (17.5 cm CRL) (Fig.35). Collagen fibers were arranged in a wavy manner with spindle shaped fibroblast and with increased quantity of reticular fibers in 83.5 days (19.5 cm CRL) embryos. Very few collagen fibers were found at 90 days. Many reticular fibers were present in fetuses aged 107 days.

Tanvi *et al.* (2015) visualized that mesenchymal cells differentiated into the fibroblasts and secreted few reticular fibres mainly towards the periphery of the capsule. They reported increase in relative amount of reticular fibres at 48 days (CRL 7.5 cm) and 50 days (CRL 7.6 cm) of gestation. Collagen fibres were arranged in thick bundles at 96 days of gestation (CRL 19.0 cm). The stromal elements at this age were composed mainly of densely arranged mesenchymal cells especially in the medulla. Large aggregations of nerve fibres were noticed in the stroma.

5.3 Histochemistry

5.3.1 Neutral mucopolysaccharides

Group I embryos at 44 days (5 cm CRL) age showed PAS- negative reaction in renal corpuscles and tubules. In Group II embryos at 96 days (24 cm CRL), there was a mild reaction in the epithelial cells lining the ducts and tubules at luminal surface. In group III at 101 days (26 cm CRL), the capsule and tubules showed mild PAS-positive reaction and the cytoplasm of collecting ducts showed intense PAS- positive reaction indicating the neutral mucopolysccharides in the collecting ducts. Basement membranes of the renal

corpuscle, tubules and collecting ducts were positive for PAS. These findings were in agreement with the findings of Gopinath and Singh (1998) who reported reduced affinity of renal tubules and glomeruli for eosin and PAS stains in 33 mm CRL goat embryos (45 days). They observed faint PAS- positive activity in the epithelial cells lining the renal tubules. The epithelial cells in the collecting ducts of all orders possessed PAS-positive granules with more aggregation in the infranuclear part of ducts of medullary area. Heavy PAS- positive reaction was observed in the epithelium of larger ducts, both in the cortical and medullary areas from 180 mm CRL onwards (96 days embryo). The cytoplasm in the cells of macula densa showed mild to moderate PAS-positive granules at 107 days of gestation. The epithelial cells lining the proximal convoluted tubules of goat foeti showed PAS- positive brush border at free surface at 250 mm CRL (112 days foetii). The intensity of PAS reaction in the epithelial cells of proximal and distal convoluted tubules increased in 290 mm CRL foeti (130 days) and beyond. PAS-positive granules were larger in the infranuclear zone of epithelial cells lining the collecting ducts.

A weak reaction of neutral mucopolysaccharides was observed in the capsule and glomeruli whereas moderate reaction was observed in the proximal convoluted tubules (PCT) in buffalo foeti at 11.2 cm CRL (Suman and Bansal, 2007). Distal convoluted tubules (DCT) and Henle's loop did not reveal any reaction. The excretory ducts in the medulla showed mild to moderate PAS-positive reaction. Capsule, glomeruli and PCT showed moderate to strong PAS-positive reaction whereas the distal convoluted tubules and loop of Henle showed weak reaction in 21.5 cm CRL foetii. Moderate to strong PAS reaction in the capsule and glomeruli and weak reaction in distal convoluted tubules and Henle's loop were observed in 44-89 cm foetii. A strong PAS -positive reaction was observed in the basement membrane and striations (brush border) of PCT in 89 cm CRL buffaloe foeti.

5.3.2 Acid and Alkaline Phosphatases

From group I to group III, the renal corpuscles, tubules and collecting ducts showed negative reaction for both acid and alkaline phosphatases. However, Davies and Davies (1952) reported that luminal surface of the mesonephric proximal tubules and metanephros showed intense positive reaction for alkaline phosphatas at an early gestation period in sheep.

CHAPTER VI

6.0 SUMMARY

The study was carried out in 45 embryos and foetuses of local Nellore sheep from 20 to 150 days of gestation to explicate the development of various structures in the kidney. In group I at 20 days (0.7 cm CRL) of embryo, the mesonephros was located lateral to the testis and occupied larger part of the coelomic cavity, cranially continued by pronephros and caudally opened with the mesonephric duct into the uro-genital sinus. At 39 days (3.2 cm CRL) the mesonephros was cream in colour, appeared as elongated oval shaped structure, its cranial border was rounded and caudal border was tapered. In 39.5 days (3.2 cm CRL) embryos, the mesonephros appeared to be 0.8 cm in length greater than the metanephric kidney. The dorsal surface of both the metanephric kidneys was in contact with psoas major muscle from group I (39.5 days) to group III (150 days). The left kidney remained in contact with the primitive stomach on the cranio-lateral / antero-lateral aspect and half of the ventral surface of right kidney was in contact with very long caudate process of the liver. The metanephric kidneys of all age groups varied in colour from light to dark brown. The shape of metanephric kidneys was irregularly ovoid upto 39 days (3.2 cm CRL), smooth and covered with a thin fibro-muscular capsule, and changed to bean shape with rounded extremities at 41 days of gestation (4 cm CRL). The size of the metanephric kidney gradually increased from group I to group III foetuses.

In 20 day (0.7 cm CRL) old sheep embryo, at the level of body somite, ventral to the intermediate cell mass towards the dorsal median line, the pronephros was placed on either side of the dorsal aorta and was surrounded by undifferentiated mesenchymal cells. It consisted of only one giant glomerulus of 225 ± 1.3 μm in size, which was placed medially and varied from middle to ventral in its location. Discontinued irregular capsular space was noticed in and around glomerular corpuscle and was lined by squamous to cuboidal cells.

The tubules present at dorsal end were elongated and irregular in shape, which traversed towards the caudal part of the vesicle. Cuboidal cells lined these tubules. A thin layer of connective tissue capsule, which was lined by squamous to cuboidal cells surrounded the pronephros at 24 days (1.1 cm CRL) of gestation. The mesenchymal differentiation progressed than the earlier age. The number of tubules was 6 to 7 and they were restricted to caudal end. Degenerative changes were noticed in glomerular network due to which vacuolation was noticed between the tuft of glomerulus at this age. The degeneration of the tubules was observed at cranial to caudal pole by 25 to 27 days (1.5 cm CRL to 1.7 cm CRL) and it was completed by 31 days (2 cm CRL) of gestation.

In 20 day (0.7 CRL) old embryos, the mesonephros was globular in shape and occupied the dorsal part of coelomic cavity from below the level of somites and cranially adjoined to the pronephros by primary excretory duct. The parenchyma was densely packed with tubules, migrating angioblasts and differentiating glomerulus. The capsule was lined by squamous to cuboidal cells. The mesonephros showed giant glomeruli, each of which filled nearly whole of the Bowman's cup by leaving a small narrow space. Most of the irregular tubules were differentiated through the condensation of mesenchymal cells, some were epithelialized and formed vesicles with narrow and wide lumen in the parenchyma. At 24 days (1.1 cm CRL) the mesonephros was surrounded by a thin capsule of 14 ± 1.2 µm that consisted of connective tissue fibers. Both the cranial and caudal parts of mesonephros were differentiated and Bowman’s/capsular space was clearly noticed. The width of the capsular space was 14 ± 1.2 µm. Visceral and parietal layers surrounding glomeruli were demarcated at this age. They were lined by simple squamous epithelium. In the visceral layer, very few cells between the squamous cells were modified and presented with large vesiculated nucleus representing the future podocytes. Most of the tubules in the parenchyma showed histological differentiation into the proximal and distal segments at

this age. Thickness of the capsule was increased to 23 ± 1.4 µm and glomerulus and tubules drastically increased in number by 27 days (1.7 cm CRL). The manifestation of macula densa of DCT, Juxtaglomerular cells of afferent arteriole and mesangial cells at vascular pole of Bowman's capsule was observed at this age. At 31 days (2 cm CRL), the proximal tubules possessed narrow lumen with brush borders. In 35.5 days (3 cm CRL) embryos, there were striking similarities in structure between the mesonephros and metanephros but there was no demarcation of cortex and medulla and loop of Henle was lacking. At 39.5 days (3.5 cm CRL) typical elongated shuttle shaped mesonephros was present medial to the metanephric kidney by showing medial concave and lateral convex surfaces. The cranial tubules lost their epithelial cell lining whereas, the caudal tubules were still developing. At 49 days (7 cm CRL) of gestation, the degenerative process was progressively increased than the earlier ages at the anterior pole. Shrinkage of glomerulus was noticed and the parietal layer of glomerulus was thickened with increased capsular space. At 74 days of gestation, the mesonephros was covered by a thick capsule which was lined by squamous cells and became as ductus deferens of the testes in this age.

The ureteric bud was formed by the evagination of caudal part of the mesonephric duct in sheep embryo at 20 days of gestation (0.7 cm CRL). The ureteric bud elongated cranially and the tip was ampullated and capped by nephrogenic/metanephric blastemal cells in 24 days sheep embryos (1.1 cm CRL). During 25 to 30 days of gestation (2 cm CRL), the branching of ureteric bud was completed and the metanephric blastema was fully transformed to rounded cap like structure. The mesenchymal cells bordering whole of the nephrogenic mass condensed at periphery as primordial capsule of the developing metanephric kidney in 29 days of embryos (1.9 cm CRL). In 37.5 days embryo (3 cm CRL), the glomerular masses increased in number than earlier ages and were suspended between the tubules and centre of the gland. The metanephric tubules occupied throughout

the periphery and centre. Metanephric kidney was divided into dark coloured cortex and lighter colour medulla at 44 days (5 cm CRL) of gestation. Renal corpuscles increased in number and were distributed towards periphery from the pelvis. Demarcation between proximal and distal tubules was noticed at this age. Major and minor calyces were apparent by 57 to 60 days and the quantity of collagen increased at the sinus around the major and minor calyces. Renal columns were more evident in 71 days than earlier age. At 78 days, the capsular space was 12 ± 2.1 μm and was reduced from the earlier ages. Loop of Henle first appeared at 83.5 days and were well established in juxta medullary nephrons as descending and ascending limbs. In group III, the typical cortex and medulla was differentiated as adult kidney and renal corpuscles were still under differentiating stages in the cortical region by 101 days. Papillary ducts were lined by stratified cuboidal epithelium, minor calyx was lined by transitional epithelium like adult kidney and smooth muscle were also noticed in the wall of the calyx minor in 115 days of gestation. By 120 days, the Juxta glomerular cells exhibited the cytoplasmic granules.

In group III at 101 days (26 cm CRL), the capsule and tubules showed mild PAS positive reaction and the cytoplasm of collecting ducts showed intense PAS positive reaction indicating the presence of neutral mucopolysccharides in the collecting ducts. The renal corpuscles, tubules and collecting ducts showed negative reaction for both acid and alkaline phosphatases in group I to group III embryos.

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