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Histological Structure of Kidney at Term Stage of Embryonic Development in Leaf Nosed Bat *Hipposideros speoris* (Schnider), Chiropteran; Mammalian

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Abstract: The kidney of early term embryo of *Hipposederosspeoris* with a body mass 0.2g and CR length 14mm, shows differentiating cortex, medulla and a developing papilla surrounded by the calyx, which leads into the ureter at the region of wide hilus.The kidney of *H. speoris* at late term pregnancy (with a body mass 2g) characterized by the well differentiated cortex, medulla and papilla. In the cortex well developed Bowman's capsules with the juxta-glomerular apparatus surrounded by the proximal and distal convoluted tubules are observed. The columnar cells lining the proximal convoluted tubules possess the differentiatingmicrovillus brush border. The distal convoluted tubules have wide lumen lined by the cuboidal epithelial cells. Medulla exhibit the differentiation of the uriniferous tubules viz., proximal tubules, thin loop of Henle, distal tubules and collecting tubules;with varying external and luminal diameter and different cell types. The large collecting ducts of the papilla open into the calyx, which leads to the ureter. Large number of interstitial cells is also observed in the connective tissue of the papilla.

Key words: Bat · Embryo · Histology · Kidney · RenalTubule

INTRODUCTION

The excretory system of mammals composed of the kidney proper, the ureters and a bladder with accessory structures; which is involved in filtration of toxic substances, removal of excess materials, conservation of water and maintenance of electrolyte balance and hence provides an optimal state conducive for metabolism through the body tissues as well as within intercellular spaces; thus maintain homeostasis. The excretory organs are paired structures and are concerned in collecting waste [1]. A strong relationship between mammal's ecological distribution and urine concentrating ability has been established in several mammals [2]. In mammals the factors such as diet and other aspects of an animal's life history influence renal specialization [3, 4]. Similar adaptations of medulla and renal papilla are observed in some other carnivores, insectivores, marsupials, rodents and Neotropicalmicrochiropteran bats [2, 5, 9].

As reported in other mammals the kidneys of chiropterans also exhibit the structural adaptations influenced by the habitats and feeding habits [5, 10, 12]. The kidney of vampire bat, *Desmodus rotundus* and *Pizonyx vivesi* exhibit the long renal papilla especially an adaptation for water conservation which is associated with their protein rich diet [7, 10, 11]. The old world fruit bats (Order- Megachiroptera) possessed kidneys with thick cortex; a thin or undivided medulla and a very short conical papilla; while the neotropical bats with animalivorous feeding habits possesses kidneys with relatively thin cortex, a thick medulla divided into outer and inner zones and a long papilla.

The development of kidneysat 21 somite stage embryo of *Rhinolophus hipposideros* and on older embryos of *Nyctalus* [= *Vesperugo*] *noctula*were noticed by Van der Strict [13]. The fetal furrows on the outer surface of embryonic kidneys of *Myotis* and *Plecotus* were observed by Sperber [2]. He also reported that, in these embryonic stages the kidneys shows no

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differentiation of cortical and medullary components. The histochemistry of protein and glycogen in the prenatal, postnatal kidneys of Indian fruit bat *Rousettus leschenaulti* and the glycogen activity in the kidneys of postnatal sucklings of two species of bats, *Megaderma lyra lyra* (carnivorous) and *Hipposederosspeoris* (insectivorous) examined by Patil and Janbhandu [14, 15]. The functional histology is the area of interest, especially when employed or combined with ultrastructural cell biology, cytochemistry or histochemical visualization [8]. The structural and cellular details of metanephric kidneys atterm stages of *Hipposideros speoris*are examined in this report.

MATERIALS AND METHODS

The Kidneys of preserved embryos of *Hipposederosspeoris* at early and late term stages of embryonic development were examined. The pregnant females of were previously collected from underground dilapidated dark rooms of old fort at Ballarshah, Maharashtra, India. The colonies comprised of hundreds of bats. The bats were collected with the help of a butterfly net; the animals were clinically anaesthetized and dissected to remove embryos. The body weight and crown rump length (CR) of selected embryos at term stageswere recorded, the kidneys were lifted, measured and then fixed in different fixatives: Alcoholic andaqueous Bouin's, 10% Formalin for 24 hours. The fixed kidneys were washed overnight in running tap water and dehydrated by passing through different grades of ethyl alcohol, cleared in xylene and embedded in paraffin (58-60°C). The sections of kidneys were cut at 5-7µm with the help of rotary microtome. For routine histology Haematoxyline-Eosin technique was used. The stained sections were observed under light microscope. The weight of embryos and the kidneys were taken by electronic weighing machine. The measurements of micro-structures were calculated with the help of ocular micrometer scale.

RESULTS

The embryo of *Hipposederosspeoris* at early term stage with a body mass 0.02g and CR length 14 mm (Fig. 1) shows that the kidney is differentiating into cortex and medulla. Development of a papilla surrounded by the calyx which opens into the ureter at the wide hilus is observed (Fig. 2).

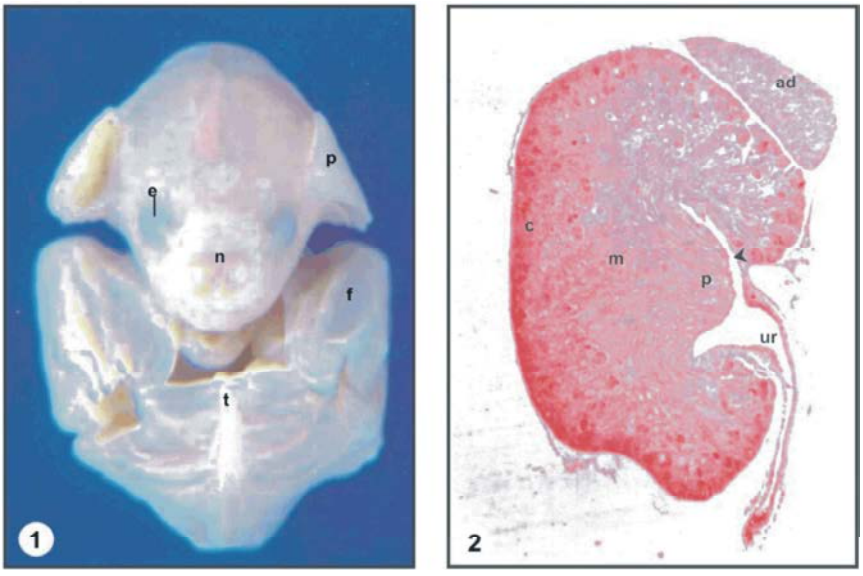

Fig. 1: Frontal view of the male prenatal embryo of *H. speoris* with a body mass 0.02g and CR length 14 mm. e: eye; f: fore limb; n: leaf nose; p: pinna; t: tail.X5

Fig. 2: Midsagittal section of the right kidney of the prenatal embryo shown in figure 1 showing the differentiating cortex (c), medulla (m) and papilla (p) which leads into the calyx (arrowhead). The ureter (ur) originates from the calyx and emerges outside the kidney through the wide hilus. ad: adrenal (H&E; X60)

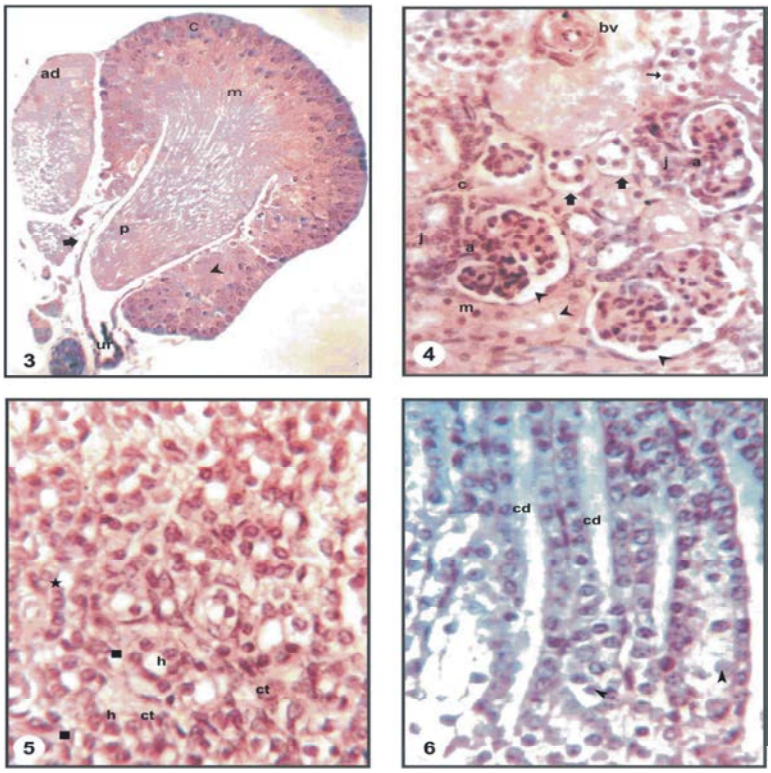

Fig. 3: Midsagittal section of the right kidney of the late prenatal embryo of *H. speoris* with a body mass 2g. showing the metanephors differentiated into a thin outer cortex (c) characterized by the darkly stained Bowman's capsules (arrowheads), the thick medulla (m) with the longitudinal sections of uriniferous tubules and a large conical papilla (p) which protrudes outside the kidney surrounded by the calyx (thick arrow). ad: adrenal; ur: ureter. (H&E; X45)

Fig. 4: Part of the figure 3 magnified to show the histological details of the cortex. Note the lobes of developing glomeruli in the Bowman's capsule. The mesangial cells with darkly stained nuclei lie in between the glomerular capillaries. The glomerular lumen (arrowheads) lined with flattened epithelial cells. The differentiating proximal convoluted tubules (thin arrows) and the distal convoluted tubules (thick arrows) are lying in the dense connective tissue. a: afferent arteriole; bv: cortical blood vessel; j: juxta-glomerular apparatus (H&E ;X450)

Fig. 5: T.S. of medulla of late prenatal kidney of *H. speoris* showing the different types of uriniferous tubules; thelarge collecting tubules (ct) with wide lumen, small tubules of the thin loop of Henle with wide lumen (h), the proximal (★) and the distal tubules (■) with intermediate diameter. (H&E X650)

Fig. 6: Papilla cut longitudinally showing large collecting ducts (cd) with wide lumen lined by the cuboidal epithelial cells with large round nuclei. Arrowheads point towards the interstitial cells with large nuclei (H&E; X460)

Both the kidneys of *H. speoris* at late term embryonic stage with a body mass 2g measures 3x2.5mm in length and width. While the weight of right and left kidneys were 0.0049g and 0.0055g respectively. The right kidney examined was well differentiated into cortex, medulla and papilla (Fig. 3). In the cortical region well-developed Bowman's capsules with well differentiated glomeruli were noticed (Fig. 4). In the Bowman's capsules the glomerular cells with round nuclei occupy the spaces in between the glomerular capillaries, which are surrounded by wide lumen lined by the squamous epithelial cells. The afferent glomerular arteriole and darkly stained juxta glomerular apparatus are observed at the vascular pole of the Bowman's capsules. Around the Bowman's capsules the renal tubules can be differentiated into the proximal and distal convoluted tubules. The proximal convoluted tubules have a narrow lumen, are lined by the columnar epithelial cells with central round nuclei and possess the microvilli protruding into the lumen. The distal convoluted tubules have wide lumen and are lined by the cuboidal epithelial cells with central, round, dark nuclei (Fig. 4). The medulla consists of the different types of the

uriniferous tubules with varying outer and luminal diameter and different cell types, viz., (i) the large collecting tubules with wide lumen lined by the cuboidal epithelial cells with central, round nuclei. (ii) The small loop of Henle with very narrow lumen lined by the squamous epithelial cells with spherical nuclei. (iii) The proximal and the distal tubules with intermediate diameter to that of collecting tubules and the thin loop of Henle (Fig. 5). The distal tubules possess comparatively wide lumina than that of the proximal tubules. At this stage the papilla is small and consists of large collecting ducts with wide lumen lined by the columnar epithelial cells with dark round nuclei. Numerous large interstitial cells are observed in the connective tissue of the papillary region. The collecting tubules and the thin loop of Henle are also observed in the papilla (Fig. 6). At this stage of embryogenesis the uriniferous tubules were surrounded by the connective tissue matrix with a network of renal capillaries. The collecting ducts open into the wide calyx, which is also lined by a layer of epithelial cells.At term stages the fetal kidneys of *H. speoris* show largest conical papilla surrounded by calyx, the papilla protrudes from wide hilus (Fig. 3).

Note:

- Measurements of different parts of Right Kidney of *H. speoris* at late term stage is given in Table 1.
- Diameter of different parts of theuriniferoustubule, ureter, renal artery and renal veinis given in Table 2.
- Details of cells in different Parts of the uriniferoustubule are given in Table3.

Table 1: Measurements of different parts of Right Kidney of *H. speoris* at late term stage

Sr. No.	Part of Kidney	Height/Length*	Thickness/Space*/Diameter**
1	Collagenous Capsule	--	96.43
2	Cortex	--	394.45
3	Medulla	--	676
4	Papilla	966*	--
5	Calyx	--	96.6*
6	Hilum	--	644**

Table 2: Diameter of different parts of uriniferoustubule,ureter, renal artery and renal vein (in µm)

Sr. No.	Part of Renal Tubule/Ureter/ Renal Artery / Renal Vein	External Diameter/Thickness*	Luminal Diameter
1	Bowman's Capsule	62	--
2	Glomerulus	53.8	--
3	J-Glomerular apparatus	16.4*	--
4	Proximal Tubule	21	3.22
5	Thin Loop of Henle	12.5	3.5
6	Distal Tubule	22.27	8
7	Collecting Tubule	28	13.6
5	Collecting Duct	32.29	16
8	Ureter	257.6	144.9
9	Renal Artery	41	12.5
10	Renal Vein	91.5	44.6

Table 3: Details of cells in different parts of uriniferoustubule

Sr. No.	Part of Renal Tubule	Diameter of Cells in µm	Diameter of Nucleus in µm	Shape of cells	No. of Cells in T.S.
1	Glomerulus	4.8	3.6	Irregular	--
2	J-Glomerular Cells	3.2	1.6	Spherical	--
3	Macula Densa	6.44	4.8	Cuboidal	5
4	Proximal Tubule	8.58	4.6	Columnar	5 to 6
5	Thin Loop of Henle	4.8	3.2	Flattened	3 to 4
6	Distal Tubule	8.37	4.7	Cuboidal	6
7	Collecting Tubule	7.2	4.83	Cuboidal	6 to 10
5	Collecting Duct	8.9	5.56	Cuboidal	9 to 13
8	Interstitial Cells	6.44	4.83	Spherical	--

DISCUSSION

In mammals the urinary system is composed of the paired kidneys, ureters and a urinary bladder for temporary storage and urethra open outside the body. The embryonic kidneys of *Hipposederosspeoris* are typically bean shaped, but the right kidney is broader towards the anterior side. Shew [16] reported similar variation in shape of the two kidneys in *Pipstrellusjaranicus*. In the embryonic kidneys of *H. speoris*studied; the renal papilla protrudes outside the hilus like other neotropical bats *Pipistrellusjaranicus* [6, 7, 16]. The chiropteran kidney is simple and is divided into an outer cortex, a single medulla converging to the terminal pyramid of papilla [17]. The differentiating thin cortex, a large medulla and the long papilla of the developing kidney in *H. speoris* indicates that length of loop of Henle is comparatively more; which is an adaptation to concentrate the urine as it passes across the long loop of Henle [18, 19]. The interstial cells present in the medullary and papillary region may act as bridges in between the uriniferous tubules and the blood capillaries [20]. It is presumed that these cells might be acting as temporary reservoir for the substrates. The tissue fluid around the blood capillaries and nephric tubules are responsible for transport of substrates across the kidney tissue.

At the early term stage examined here the kidneys were not differentiated into cortical and medullry components; this differentiation was well demarcated in the kidney of late term stage. The renal medulla of late term embryo was not further divided into zones at this stage of development; a condition reported in other insectivorous bat species [21-23]. Likewise the different parts of renal tubules, viz., the proximal tubules, distal tubules, collecting tubules and collecting ducts were not differentiated in the early term stage; while the various parts of renal tubules were completely differentiated in the kidneys of embryo at late term stage. The external surface of kidneys examined, shows typical fetal furrows, as seen in*Myotis* and *Plecotus* [2]. Patil and Janbhandu [24, 25] examined the protein and carbohydrate histochemistry in the embryonic kidneys of *Megaderma lyra lyra* and *Hipposederosspeoris*. The dark protein and carbohydrate reactions in the brush border of epithelial cells of proximal tubules suggesttheuriniferous tubulesare preparing for the function of filtration as well as absorption of substances by diffusion. The urine formation mainly involves glomerular filtration, tubular reabsorption and tubular secretion. The dense microvillusbrush border facilitates more reabsorption of glucose, phosphates, minerals etc. The thick medulla and the papilla indicate the long of long loop of Henle; the urine becomes more concentrated as it passes along the loop of Henle [9]. In the embryonic kidneys of *Megaderma lyra lyra* [26] the large papillary cavity at phalange stage was noticed; such structures was not seen in *Hipposederosspeoris*.

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