HISTOLOGICAL STUDIES ON EPIDIDYMIS OF INDIAN FRUIT BAT, ROUSETTUS LESCHENAULTI DURING THE REPRODUCTIVE CYCLE

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Abstract: The present paper deals with changes in the epididymis of Indian fruit bat, *Rousettus leschenauliti* during the sexually active- inactive periods.The epididymis of this bat is divided into three regions i.e.the caput ,the corpus and the cauda epididymis. The tubules of the epididymis showed variation in the diameter,height of the epithelium, cell types ,number of spermatozoa and interconnective tissue between the tubules during the sexually active –inactive periods. The epididymal epithelium is composed of principal cells, basal cells and apical cells .The role of epididymis in transport, maturation and storage of spermatozoa is discussed.

Keywords: Epididymis, Bat, Reproductive Cycle.

Introduction: The epididymis is a highly convulated tubule which connect the testis to the ductus deferens and is an important segment of the excurrent duct system of the testis that performs a variety of functions. The epididymal duct is not a mere sperm transporting duct; it is an androgen dependant organ, which play a key role in the transport, maturation and storage of spermatozoa. The epididymal epithelium is responsible for creating the ideal conditions that make spermatozoa fertile and motile via absorption, secretion, synthesis and metabolic activity. Structure of mammalian epididymis has been described by many workers (Crichton and Krutzsch, 2000; Aguilera-Merlo *et al*,2005: Domeniconi et al, 2007). Considering the worldwide distribution and immense diversity exhibited by members of the order Chiroptera, remarkably limited attention has been given to reproduction in the male.There are many gaps in our basic knowledge of the morphology and physiology of the male reproductive system of Chiroptera . Amongst bats, structure of epididymis has been reported in few species of bats (Gopalakrisshna et al,1974; Sapkal and Sahasrabudhe,1986; Beguelini *et al*,2010; Shende, 2013).

The present study has therefore, been undertaken with a view to examine histoarchitecture of epididymis of *R. leschenaulti* during the reproductive cycle and to find out possible role of the epididymis in the process of sperm transport and maturation in this species of bat.

Materials and Methods: The Indian fruit bat, *R. leschenaulti* is selected for the present study because of its unique reproductive habits. Gopalakrishna and Choudhari (1977) gave an account of sex cycle of the Indian fruit bat, *R. leschenaulti*. It is seasonally dioestrous tropical species (Bimodal breeding sequences), which breeds twice in a year. The first peak period occurs during October-November and the second during February-March. The male reproductive organs show slight regression in January but become active in February and March. After the second pregnancy start, the male organs start regression and are in an inactive state from May to September.

The male specimens of the Indian fruit bat, *R. leschenaulti* were collected throughout the year representing all the reproductive states from an underground tunnel of Kandri Mine, Nagpur in Maharashtra State, India . The specimens were netted at random with the help of butterfly net. These animals were brought alive to the laboratory with minimum stress and constant supply of food and glucose water. Live animals were at once anesthetized with anesthetic ether and the some specimens were weighed immediately at the sensitive spring balance, to keep the body weight record and then killed by decapitation. Males weighing 68 gms and over are sexually mature. The highest body weight recorded of a male was 115 gms.

For the present study the testis and epididymis were dissected out and fixed in aqueous Bouins for histological study. After fixation for 24 hrs, tissues were washed with water and dehydrated through the graded series of ethanol, cleared in xylene and embedded in paraffin wax. Thin sections of 3-5µ were cut with the help of Leica 2417 microtome. Testis and epididymis were stained with Ehrlich"s Haematoxylin–eosin for histological observations. The micro-measurements were taken with the help of an ocular micrometer caliberated to a stage micrometer. The photographs were taken with the help of Karl Zeisss camera attached to microscope and enlarge to the required size.

Observations: The epididymis of *Rousettus leschenaulti* can be recognized into three distinct regions-anterior caput epididymis, middle corpus epididymis and posterior cauda epididymis. Caput epididymis forms a cap on the anterior end of testis. Corpus epididymis is narrow tube which lies along the dorso-median border of testis. Cauda epididymis is situated on the posterior end of testis. In active breeding period the cauda epididymis is slightly bigger than the caput epididymis in adults. From the antero–ventral end of cauda epididymis arises the vas deference which traverses through inguinal region and opens into the distal end of the tubular part of seminal vesicle of the respective side.

Epididymis during the Sexually Inactive Period:
Caput Epidiymis: Epididymal tubules are very small in size, diameter is very less as compared to diameter during the other reproductive phases of animal. Connective tissue present in between the tubules is very thick. The epididymal tubules are lined by cuboidal epithelial cell rest on basal lamina Three different types of cell are clearly visible. These are principal cell, basal cells and apical cells. The Principal cells are cuboidal and contain darkly stained round nuclei situated towards the center of the cell. Nucleolus is single and centrally situated. Cytoplasm is eopsinophillic .From apical surface of cell microvilli or stereocilia are seen projecting into lumen. The Apical cells are small sized cells. They are very few in number. They are found lying very close to lumen. Nucleus is round and darkly stained, cytoplasm is eosinophillic. The basal cells lie very close to basal lamina, just below the principal cells. They are small sized. Nucleus and cytoplasm is darkly stained(Fig. 1).

Cauda Epididymis: Cauda epididymal tubules are small in size compared to other phases. Thick connective tissue is present in between the tubules. The tubules are lined by single layer of cuboidal epithelial cells. The principal cells show round prominent and darkly stained nuclei. One or two nucleoli are seen in the nucleoplasm. Microvilli are found projecting into the lumen. Lumen shows some residual secretion (Fig. 2). The basal cells and apical cells show similar characteristic as in caput epididymis.

Epididymis during the Active Breeding Period
Caput Epidiymis: Diameter of the epididymal tubules is increased during the breeding period as compared to inactive phase. Size of lumen is also increased. Epithelial cells of epididymal tubule are resting on basal lamina. Intertubular connecting tissue is thin and shows blood vessels. The lumen contains some secretory material. The tubular epithelium is made of different types of cells. The Principal cells are tall columnar with prominent and darkly stained oval to spherical centrally located nucleus. Cytoplasm is eosinophillic.Stereocilia are seen on the apical surface of the cell. The apical cells are small sized and very few in number. Nucleus is round and darkly stained. Cytoplasm is eosinophillic. They lie in between the Principal cells. The basal cells are located below the principal cells, very close to basal lamina. These are small sized cells. Nucleus and cytoplasm are darkly stained (Fig.3).

Cauda Epididymis: During the active breeding period epididymus attain the maximum size. Diameter of epididymal tubules is increased as compared to cauda epididymis of the previous period. Height of the epithelial cells and diameter of lumen are increased as compared to inactive phase. Intertubular tissue is thin and blood vessels are seen in the connective tissue. Epididymal tubules are lined by principal cells. They are columnar epithelial cells resting on basal lamina. Nucleus is oval and darkly stained. One or two nucleoli are also visible in nucleoplasm. Cytoplasm is eosinophilic. Apical part of cells shows stereocilia. There are few apical cells present near the luminal border. The lumen of both the caput and cauda epididymis is filled with sperms and secretion. Eosinophilic cytoplasmic droplets are observed is caput epididymis (Fig.4).

Discussion: The epididymis is essential for normal reproduction of eutherian mammals because sperm leaving the testes are incapable of fertilizing the oocyte. Testicular sperm lack mobility and fertility but they acquire these abilities by the time they reach the tail of the epididymis . The acquisition of these abilities by sperms

depend upon their passage through specific environment, which in turn is regulated by the absorption and secretary activities of the epithelial lining the ducts of the testes (Andonian and Hermo, 1999).

Studies on the mammalian epididymis have shown that this organ can be divided into distinct regions according to the biochemical, morphological and morphometric characters of its segments. Various divisions have been proposed and the most widely used is that dividing the organ into three major segments, the head (caput), the body (the corpus), and the tail (the cauda) epididymis (Dhamani, 2003; Beguelini *et al*, 2010). The epididymis of bat, *Rousettus* is divided in to three regions. Caput, corpus and cauda as in mammals and other species of bats. However, the initial segment in addition to caput ,corpus and cauda in six species of bat has been recognized (Beguelini, et. al., 2010). Sapkal and Sahasrabudhe (1986) studied the epididymis of bat, *Rousettus leschenaulti* and revealed seven regions with seven types of tubules in the caput epididymis

The mammalian epididymis is lined by a pseudostratified epithelium and different cell types have been described in epithelium in various species of mammals (Robaire and Hermo, 1988). These cells have been presumed to have different functions (Robaire and Hermo, 1988). The pseudostratified columnar epithelium of epididymis composed of four main cell types. The epididymis of neotrophical bats, *Eumpos glaucinus* and *Molosus molosus* shared a pseudostratified columnar epithelium with extensive stereocilia. It is composed of three main cell types- apical, basal and principal cells. The other cell types, clear and halo cells, were also observed, but in smaller number and only in some segments (Beguelini, et. al., 2010).The presence, abundance and localization of each cell type is varied between the species. As in the majority of mammals, the principal cells was the pre dominant cell type in all segments and their abundance varied little between the epididymal segments of *Rousettus*, similar to that reported in the bat (Beguelini, *et. al*., 2010 and shende,2013).

Stereocilia of various length and structural complexity have been identified as common projection of apical surface of principal cells in the epididymis (Schimming and Vicentini, 2008). The functional significance of the bush border is unclear but probably it conveys the luminal content forward as in mice (Orsi *et al*., 1998). During the active breeding period of this species of bat ,there is an increase in tubular diameter and epithelial cell height as compared to the inactive period. Similar observations are reported in other species of bats comfirming our results.(Gopalakrishna *et al*, 1974; Beguilini *et al*, 2010; Shende, 2013).

Rodríguez, *et al*, (2015) in bat (*Corynorhinus mexicanus*) ephasized upon the role of sperm storage in epididymis which allow the activation of proteins involved in flagellar movement of sperms. Thus, it is assumed that, protein synthezing machinery in the bat epididymal epithelium may be responsible for maintaining the microenvironment of the lumen which helps in acquiring mobility and fertility when sperms passes through the epididymal duct of bat, *Rousettus*.

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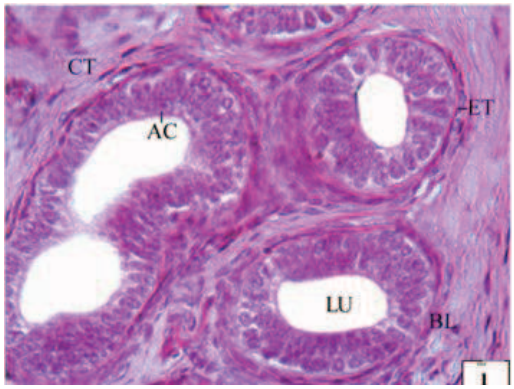

Figure 1

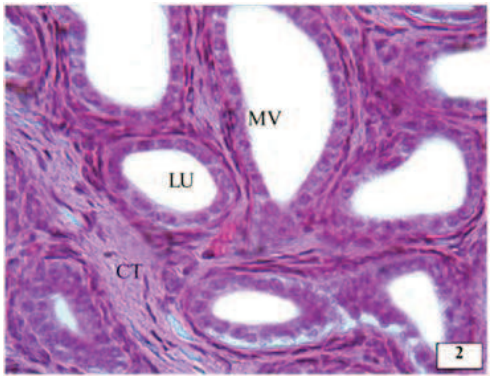

Figure 2

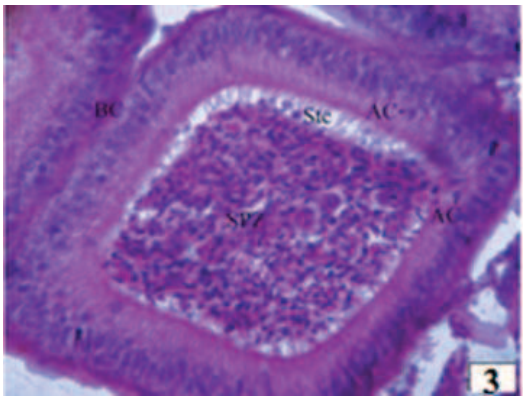

Figure 3

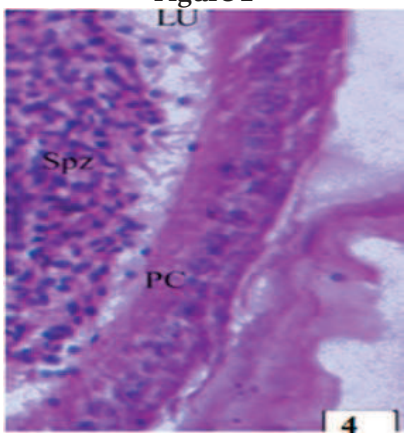

Figure 4

Fig. 1: T.S. section of caput epididymis during sexually inactive period showing epididymal tubules (ET) lined by different types of epithelial cells resting on basal lamina (BL), the principal cells with prominent nucleus, one or two nucleoli and few basal cells are seen. Stereocilia (stc) are seen on the outer surface of cells. (X400)

Fig. 2: T. S.of cauda epididymis during sexually inactive period shows, Epididymal tubules (ET) surrounded by thick connective tissue (CT). A single layer of epithelial cells is resting on basal lamina (BL). Principal cells show, round darkly stained nucleus (N), one or more nucleoli and lightly stained cytoplasm. (X600)

Fig. 3: T. S.of caput epididymis during sexually active period shows, columnar principal cells (PC), and apical cells (AC), with clear lightly stained cytoplasm. Small basal cells (BC) with small nuclei are found attached to the basal lamina. (X400)

Fig. 4: T. S. of cauda epididymis during sexually active period shows epididymal tubules (ET) separated by moderate connective tissue (CT). Columnar principal cells (PC) are found resting on basal lamina (BL). Very few basal cells (BC) and apical cells (AC) are seen. Stereo cilia are seen on the surface of cells. Lumen (L) contains large number of spermatozoa (SPZ). (X400)

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