HISTOLOGICAL STUDIES OF INTERSTITIAL CELLS IN THE OVARIES OF BATS *HIPPOSIDEROS SPEORIS* AND *ROUSETTUS LESCHENAUTI*

Nitin P. Meshram¹ and Kishor S. Janbandhu²

¹S. K. Porwal College, Kamptee, Nagpur [India]

²Govt. Institute of Science, Nagpur [India]

mnitin_p@yahoo.co.in

Abstract:

Bats are dominating flying mammals all over the world showing various adaptive specialization. Chiroptera shows great variations in the reproductive stage, even the closely allied species exhibit different breeding habits. It is important to study the reproductive organ that is ovary in respect with the interstitial cells. Because the interstitial cells with the help of LH of pituitary produce a hormone androgen. That means interstitial tissues are developed as a gland having steroidogenic activities. In the immature ovary of *Hipposideros speoris*, interstitial cells are not distinguished from the stromal cells while it is clearly distinguished from stromal cells in the immature ovary of *Rousettus leschenaulti*. In immature ovary of *Rousettus leschenaulti* numerous interstitial cells observed in the stromal area. In the inactive *Hipposideros speoris* ovary, bundles of interstitial cells observed 60 ± 5 . In the inactive ovary of *Rousettus leschenaulti* interstitial cell bundles observed 41 ± 5 . Number of interstitial cells in each bundle observed in inactive ovary of *Hipposideros speoris* are 5 to 10 and in *Rousettus leschenaulti*, it is observed 5 to 7. In the active ovary of *Hipposideros speoris*, the bundles of interstitial cells observed 80 ± 5 and in *Rousettus leschenaulti* it is observed 47 ± 5 . Number of interstitial cells in each bundle observed in *Hipposideros speoris* is 7 to 30. While in *Rousettus leschenaulti* observed 7 to 20. Pregnant ovary of *Rousettus leschenaulti* shows inverted corpus luteum, atretic follicles, destructed follicles and interstitial cells. So it is concluded that the interstitial cells in the active ovary is more steroidogenic than inactive ovary.

Keywords: *Hipposideros speoris*, *Rousettus leschenaulti*, interstitial cells

Introduction

India has a rich chiropteran fauna. 17 families 174 genera and 913 species of bats reported by Koopman (1994). At present 1116 recognized species of bats worldwide Simmons SB (2005) out of the 17 families, 7 families incorporating over of 100 species are reported in India.

A literature survey reveals that meager information is available on the interstitial cells in the ovary of Indian Bats. Guraya (1901), Singh and Krishna (1994), Abhilasha and Krishna (1996) *Megaderma lyra lyra* breeds end week of November and delivering in 1st to 3rd week of April. *Hipposideros* breeds last week of December to 2nd week of March, deliveries in May to mid June.

The present work includes observation on the interstitial cells in ovary of two species of Indian bats *Megaderma lyra lyra* and *Hipposideros speoris*.

Material and Methods

Female specimens of *Megaderma lyra lyra* and *Hipposideros speoris* used for present study collected from the Ballarshah and Kandri mines, near Nagpur in India. The bats were collected from delapidated dark rooms and mines with the help of mist nets and butterfly net, the collection made as per requirement. The animals killed by chloroform and their body

weight taken and fixed in 10% formalin for 24 hours.

HISTOLOGY

The female reproductive system was dissected out, their ovaries were separated and fixed in 10% formalin for 24 hours. Tissue washed overnight in running water. The tissue was dehydrated by passing through a graded series of ethyle alcohol, cleared in xylol and embedded in paraffin wax (58^o-60^oC).

The sections were cut for the thickness of 4 to 5 μ with the help of rotary microtome for routine histological study. The sections were stained with haematoxyline eosin stain.

The photographs were taken with the help of 35mm leica camera attached to the microscope and enlarged to the required size. All microscopic observations, measurements were taken with the help of an eyepiece micrometer calibrated to stage micrometer scale.

HISTORICAL REVIEW

May J. Guthrie, Katnarine R. Jeffer (1938a) have been studied the ovaries of bats *Myotis lucifugus lucifugus* and *M. grisescens* and informed that Interstitial cells very numerous in Newborn bats and they arose from hypertrophied theca interna of retrogressing follicles of granulosa cells. Mossaman et, al (1964) have recently designated that these Interstitial cells of thecal origin in the

mammalian ovary are of first type of Interstitial cells. The second type of interstitial cells in the ovary of bat are in the form of epithelial cells are irregularly distributed among the first type of interstitial cells.

Brambell FWR (1906) described that there are several type of glandular tissues in the mammalian ovaries which are severally described as Interstitial cells. The different view regarding their origin have been produced by Brambell to two comprehensive thesis namely epithelial origin and the connective tissue origin of the interstitial cells. In many species of mammals the ovarian interstitial tissue consists of cellular clusters and nests Guraya (1967) which are scattered in the cortical stroma. Investigated that ovaries of non pregnant and pregnant grey bat *Myotis grisescens* show two types of Interstitial cells. The first type of cells were considerably developed and occupy all the ovary except the periphery which contain small follicles they originated by the thecal hypertrophy of atretic large Preantral and antral follicles and they are apparently developed to the some extent in the ovaries of pregnant and non pregnant bats. The second type of interstitial cell was in the form of epithelial cells which were irregularly distributed among the first type of interstitial cell.

Sardul S Guraya, Gilbert, S Greenwald (1964) studied various mammalian ovarian interstitial tissues and described that in Rat ovary, the interstitial cells occur as scattered patches of various sizes that are derived from the theca of atretic preantral follicles and antral follicles. Most of Bat ovary, The interstitial cells occupied the total ovarian area except the cortex and the interstitial cells distributed and scattered in between the follicles and connective tissue area.

Observations

Interstitial cells in Immature ovary of *Hipposideros speoris*

The observations in immature ovary of *Hipposideros speoris* shown that the Interstitial cells cannot be distinguished from the stromal cells. The number of developing primordial follicles seen. Amongst the developing follicles small clusters of cells seen with lightly staining vesicular nuclei in some places, these are forerunner of the interstitial cells. The interstitial cells does not become well differentiated in *Hipposideros speoris* in the immature animals (fig.1a and 1b)

Interstitial cells in mature ovary of *Hipposideros speoris*

In mature ovary of *Hipposideros speoris* shows, the larger area of stroma composed of connective tissue cells with elongated nuclei. follicular composition of the ovary shows various different stages of the reproductive cycle development. In inactive ovary only primordial, unilaminar, bilaminar, preovulatory, graffian follicles, antral follicles are found.

Interstitial cells in Inactive ovary of *Hipposideros speoris*

The inactive ovary of *Hipposideros speoris* showed the polyhedral and polygonal shaped scattered interstitial cells found in between various follicles. The interstitial cells are found in small bunches, In the stromal as well as peripheral epithelial area. The connective tissue also seen in the stromal area. The polyhedral interstitial cells having vesicular nuclei. In this stage very few bunches of interstitial cell found distributed in small stromal area because the unilaminar follicles contain large in number and stromal area becomes narrow. In inactive ovary interstitial cells are small about 5 to 7 μ . About 5 to 10 cells are consisted in each bunch. There are 60 ± 5 such bunches are found in all over the ovarian stroma. (fig 2)

Interstitial cells in active ovary of *Hipposideros speoris*

The active ovary of *Hipposideros speoris* noticed that multilaminar follicles, single preovulatory follicle, follicles undergoes atresia. The stroma is compound of polyhedral interstitial cells with rounded nuclei. A few blood capillaries also supplied with interstitial cells. As the primordial follicles are few in number, stromal area becomes larger and number of clusters of interstitial cells are increases. about 80 ± 5 bunches of interstitial cells with rounded nuclei are seen distributed among the follicles in the stromal region. Each bunch consisted of about 7 to 30 interstitial cell. Interstitial cells in active ovary is more hypertrophied than inactive ovary are measured about 7 to 10 μ . (fig. 3)

Interstitial cells in Immature ovary of *Rousettus leschenaulti*

In the immature ovary of *Rousettus leschenaulti* germinal epithelium of ovary is composed of flat cells. Ovarian stroma made up of small ovoid cells and sparsely distributed between the follicles and occupy a major part of the ovary. In the large immature ovary stromal cells are elongated and spindle shaped so that the stroma appears fibrous. The connective

tissue fibres more abundant and prominent.(Fig4a,4b).

Interstitial cells in Mature Ovary of *Rousettus leschenaulti*

In the mature ovary of *Rousettus leschenaulti* the germinal epithelium is composed of cuboidal cells and interstitial cells. The tunica albuginea varies in its thickness in different region of the ovary. The stroma is compound of fusiform cells having spindle shaped ovoid shaped nuclei and irregularly distributed connective tissue fibers. small number of interstitial cells occurs in the ovarian stroma. The interstitial cells are some what larger than the stromal cells, and are polygonal or polyhedral in shape. The interstitial cells are closely packed and in contact with the neighbouring cells which any intervening tissue except for blood capillaries. blood capillaries were evenly scattered through the gland. The interstitial cells are seen in bunches. The interstitial cells are 3 to 15 in numbers in various different bunches. Blood capillaries are supplied with the interstitial cells in some places

Interstitial cells in inactive ovary of *Rousettus leschenaulti*

An animal found inactive in August to November. The peripheral part of the ovary contains a large number of primordial follicles, developed into unilaminar, bilaminar, multilaminar follicles, which reach upto a early vesicular stage of follicular development undergo atresia. All the follicles are distributed in stromal area. Made up of ovoid cells and occupy a major part of the ovary.

This animal breeds twice in the year so that pregnant females occur in all the months of the year except August to November. The breast feeding female come to heat again and undergo copulation and carry second pregnancy. So that the structure of interstitial cells is very much variable in this animal. The number and sizes of interstitial cells are vary. In inactive animal ovary shows 41 ± 5 bunches of interstitial cells in the stromal area in between the follicles. These cells are some what bigger to that of stromal cells. In each bundle there are about 5 to 7 interstitial cells measure about 5 to 10 μ . The size of interstitial cells in inactive ovary is found to be smaller than that of the active ovary and the interstitial cells is inactive ovary is less hypertrophid than that of active ovary (Fig5a,5b).

Interstitial cells in Active Ovary of *Rousettus leschenaulti*

many germinal epithelium cells are enlarged to form the epithelial nodules. Epithelial nodules are the fore runner of the future oocytes. There are not much differences in the structure of the ovarian stroma in the ovaries of sexually inactive and sexually active specimens. In both these cases the stroma is composed of fusiform cells having spindle shaped ovoid nuclei and irregularly dispursed connective tissue fibres.

Interstitial cells are occur in stromal area. These cells are polyhedral and polygonal in shape and larger than the stromal cells. These cells are supplied with blood vessels and capillaries. Interstitial cells are polygonal in shape with spherical nuclei found distributed in stromal region. There are 47 ± 5 smaller and larger bunches of interstitial cells are scattered in the pherepheral stromal area to deeper stromal area. There are about 7 to 20 number of interstitial cells in each bunch. interstitial cells are also found in the vicinity of atretic follicles. In the active ovary thecal as well as stromal interstitial cells mixed together and found dominated with stromal area. The size of interstitial cells are measure about 9 to 12μ . The size of interstitial cells in active ovary is larger than that of inactive ovary being the interstitial cells in active ovary is more hypertrophied than the inactive ovary (Fig6).

The pregnant ovary of *Rousettus leschenaulti* observed degenerating follicles and interstitial cells. One of the function of interstitial cell is to degenerate follicles when function is over (Fig7).

Discussion:-

As per the above observation it is found that the interstitial cells are found in the ovary of immature and mature animals both. In inactive and active stages of ovaries of *Hipposideros speoris* and *Rousettus leschenaulti* bats showed some what structural differences. Some observations resulted that the interstitial cells or Epithelial cords are seen throughout the reproductive cycle in the species *Hipposideros speoris*. The epithelial cords or interstitial cells originated either by the invagination of the germinal epithelium and from the hypertrophy of persistant granulosa cells of atretic primordial and preantral follicles (M.S. sasty and S.B. Pillai (2013)

In my observations it is seen that the interstitial cells found in *Hipposideros speoris* in inactive as well as active ovary, but it is not differentiated from the stromal cells in case of

immature ovary. In *Rousettus leschenaulti*, interstitial cells observed in inactive as well as active ovary and it is also differentiated from the stromal cells, in case of immature ovary. In the immature ovary of *Rousettus leschenaulti* numerous interstitial cells observed in the stromal area. interstitial cells in ovary numerous in new born bats in *Myotis lucifugus* and they are arose from hypertrophied theca interna of retrogressing follicles of granulosa cells. (Guthrie and Jeffer, 1938a). Interstitial cells of thecal origin are in the mammalian ovary (Mossaman et al, 1964). Several type of glandular tissue in the mamalian ovary which are severally described as interstitial cells. Watzka (1957) stated that the primary function of the interstitial cells tissue was nourishment and destruction of follicles and made no distinction between the thecal gland cells and the interstitial cells.

M.S. Sastry and S.B. Pillai (2013) observed the *Hipposiderous speoris* ovary and resulted that, the epithelial cords i.e. interstitial cells were observed throughout the reproductive cycle. The epithelial cords have originated either by the invagination of the germinal epithelium and from the hypertrophied of persistant granulosa of atretic primordial and preantral follicles. Further study shows that varying numbers of cords and number of interstitial cells in various developmental stages.

Antonin Bukvsky et al (2002) studied rat ovarian interstitial cells and described the 5 stages of interstitial cells development.

Immature – small (5 to 7 μm), Young-large (8 to 10 μm), Mature – large (12 to 16 μm), Aged – large (12 to 16 μm), Regressing –Shrink (5 to 7 μm) In the pregnant *Rousettus leschenaulti* destruction of follicles as well as interstitial cells found.

Figure-1a Immature ovary of *Hipposiderous speoris* showing various developing primordial

follicles. Interstitial cells not identified from stromal cells x100.

Figure-1b Immature ovary of *Hipposiderous speoris* showing various developing primordial follicles. Interstitial cells not identified from stromal cells. X400

PF- primordial follicles

Figure- 2 Inactive ovary of *hipposiderous speoris* showing large number of primordial follicles and clearly seen interstitial cell bunches distributed all over the stromal area. X400

PF- Primordial Follicles, IC- Interstitial Cells

Figure-3 Active ovary of *hipposiderous speoris* showing follicle and atretic follicles along with numerous bunches of interstitial cells distributed all over the stromal area. x600

IC- Interstitial Cells, AF- Atretic Follicle, VF- Vesicular Follicle

Figure-4a Immature ovary of *rousettus lechenaulti* showing developing follicles x100
PF- Primordial Follicles

Figure -4b Immature ovary of *Rousettus lechenaulti* showing primordial follicles and also clearly seen interstitial cell bunches in large stromal area. X400
PF- Primordial Follicles, IC- Interstitial Cells

Figure -5a Inactive ovary of *Rousettus lechenaulti* showing various developing follicles and large stromal area with interstitial cell bunches x400

Figure- 5b Inactive ovary of *Rousettus lechenaulti* showing two layered follicle, primordial follicle and interstitial cell bunches in stromal area in the periphery. X400
PF- Primordial Follicles IC- Interstitial Cells, VF- Vesicular Follicle

Figure -6 Active ovary of *Rousettus lechenaulti* showing interstitial cell bunches in stromal area along with 3-4 layered follicles. X400
IC- Interstitial Cells

Figure -7 Pregnant ovary of *Rousettus lechenaulti* showing destroying follicles and disintegrated interstitial cells alongwith major part of introverted corpus leuteum x100.
AF- Atretic Follicle, IC- Interstitial Cells, CL- Corpus Leuteum

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