

RESEARCH ARTICLE

Histochemical Study of Protein in Interstitial Cells in the Ovaries of bats *Rousettus Leschenaulti*, *Megaderma Lyra Lyra*, *Hipposideros Speoris*

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ABSTRACT

The presence of interstitial cells in the stromal region of the immature, inactive and active ovary of bat have been studied. The interstitial cells are larger than the stromal cells are polygonal in shape. Stroma and interstitial cells supplied with blood vessels. In the present study localization of protein was find out in the vicinity of interstitial cells. Histochemistry combines the methods of histology with those of biochemistry, to reveal the biochemical composition of tissues and cells. Protein serves variety of functions and acting as receptors, enzymes, conjugates, precursors and substrate for supplicating energy to the intricate events that occur in the ovary. For the localization of Protein, tissue (ovary) fixed in 10% formalin were used. The sections were cut at 4 - 6 µm. The presence of Protein demonstrated by mercuric bromophenol blue method. The hydrated sections were stained for 15 minutes and treated with 0.5 % aqueous acetic acid with 2 changes of 20 minutes. The section were dehydrated and cleaned in Xylene. The sections were treated with 100 ml Xylene and 0.5 ml n -butyl amine until they turned blue cleaned in Xylene and mounted in DPX. Protein stained blue. The distribution of mercuric bromophenol positive proteins in the interstitial cells in the ovaries of *Rousettus leschenaulti*, *Megaderma lyra lyra* and *Hipposideros speoris* bats observed in active as well as inactive condition. In inactive condition of ovary, protein staining in the interstitial cells showed moderate and in the active condition of ovary, protein staining showed high. Inactive ovary showed a positive activity of protein in interstitial cells and in most of the ovarian components. The cytoplasm of germinal epithelium cells and the stromal region was strongly stained. The primary, secondary, Graafian follicles and zona pellucida exhibited a strong stain. The corona radiata and theca folliculi showed a moderate reaction. The ovarian interstitial cells showed moderate to high reactions with mercury bromophenol blue in the pregnant ovary of all the three species of bats.

Keywords :- Protein , Mercury Bromophenol blue , Interstitial Cells, Ovary

1. INTRODUCTION

Protein serves variety of functions and acting as receptors. Enzymes, conjugates, precursors and substrate for supplicating energy to the intricate events that occur in the ovary. The presence of interstitial cells in the stromal region of the immature, inactive and active ovary of bat have been studied. The interstitial cells are larger than the stromal cells are polygonal in shape. Stroma and interstitial cells supplied with blood vessels Guraya [1], Kanako [2] studied mice ovaries and suggested that, ovaries are different from male gonads, in that steroidogenic cells are not differentiated during the fetal period. No fetal theca and interstitial gland cells with an appearance resembling fetal leydig cells.

All the three species *Rousettus leschenaulti*, *Megaderma lyra lyra* and *Hipposideros speoris* having different breeding seasons so that the species collected throughout the year. The bat was collected without disturbing their roosts from underground delapidated dark rooms, Ballarshah, Chandrapur and Kandri mines throughout the year. The species were collected with the help of a butterfly net. Animals were brought alive to the laboratory with minimum stress. Wet gunny bags rapped to the plastic cage were used to provide lowered temperature and set darkness. Their body weight was taken with the help of a spring balance immediately after anaesthetized them with mild clinical anesthesia.

Rousettus leschenaulti collected in Nov- Dec. and March- April. *Megaderma lyra yra* was collected in Nov and April. *Hipposideros speoris* were collected Dec, March and May, June.

Investigations on the various aspects of reproduction of several species of Indian bats belongings to diverse families and exhibiting different types of breeding behavior have been reviewed by Gopalakrishna and Sapkal [3], Sastry and pillai [4]. Number of scientists has done lot of work on the Interstitial cells in the bats and other mammals but a survey of literature reveals that meager information is available on the interstitial cells of these three species of bats.

Bats represent about 20 % of all classified mammals species worldwide, with about 1116 bat species divided into two suborders, the less specialized and largely fruit eating megachiroptera, flying foxes, and the more history specialized and ecolocating microchiroptera Simmon [5]. About 70% of bats are insectivores. most of

the rest are frugivores, fruit eater. A few species are of the vampire bats.

Rousettus breeds twice in a year first breeding cycle Nov-Dec deliveries in March- April. Second breeding cycle March- April, deliveries in June - July. *Megaderma lyra lyra* breeds end week of Nov and deliveries in 1st to 3rd week of April. *Hipposideros speoris* breeds last week of Dec. to 2nd week of March, deliveries in May to mid June Wimsat [6].

The present work includes detailed observations on the Protein histochemistry of the adult (Active and Inactive) and pregnant interstitial cell in the ovaries of three species of Indian bats *and Rousettus leschenaulti*, *Megaderma lyra lyra* and *Hipposideros speoris*.

2. METHODOLOGY

Histochemistry

The Animals were brought alive to the laboratory with minimum stress. Wet gunny bags rapped to the plastic cage were used to provide lowered temperature and set darkness. Their body weight were taken with the help of a spring balance Immediately after anaesthetized them with with mild clinical anesthesia. The female reproductive system was dissected out, the ovaries were separated and fixed in alcoholic Bouin's fixative, 10% formalin and Neutral formalin for 24 hours for the observation of histochemical localization of Protein. For the demonstration of protein tissue fixed in 10 % formalin were used. The sections were cut at 4 - 6 μ m and stained with mercury bromophenol blue method (Chapman [7]).

Mercury bromophenol blue reagent

Mercuric Chloride	1 g
Sodium Bromophenol blue	0.05 g
2 % acetic acid (aqueous)	100 ml.

The hydrated sections were stained for 15 minutes and treated with 0.5 % aqueous acetic acid with 2 changes of 20 minutes. The section were dehydrated and cleaned in Xylene. The sections were treated with 100 ml Xylene and 0.5 ml n -butyl amine until they turned blue cleaned in Xylene and mounted in DPX. Protein stained blue.

3. RESULTS

Histochemical observations of protein in the interstitial cells in the ovaries of *Rousettus leschenaulti*

The protein staining visualized in granulosa was investing various follicle types, theca externa and intema, interstitial tissue and stroma. This ranges from moderate to high. The granulose cells of unilaminar and bilaminar follicles showed light protein staining. The various preantral follicles show weak staining for proteins. It is observed that the protein staining moderate in the inactive ovaries of *Rousettus leschenaulti*. In the active ovaries protein staining show high. It means that when the ovary remains active, It is more secretary than that of inactive ovary. In the active ovary the amount of proteins increases.

The distribution of mercuric bromophenol positive proteins in the interstitial cells in the ovaries of *Rousettus leschenaulti* bat observed in active as well as inactive condition. In the immature ovary intensity of protein is less. The developing primordial follicles stain moderately but stromal area stain less with Protein (Fig

1). In inactive condition of ovary, protein staining in the interstitial cells showed moderate. Inactive ovary showed a positive activity of protein in interstitial cells and in most of the ovarian components. The cytoplasm of germinal epithelium cells and the stromal region was strongly stained. The primary, secondary, Graffian follicles and zona pellucida exhibited a strong stain. The corona radiata and theca folliculi showed a moderate reaction (Fig2).

In the Interstitial cells of active condition of ovary, protein staining showed high (Fig3a and3b).

The ovarian interstitial cells showed moderate to high reactions with mercury bromophenol blue in *Rousettus leschenaulti*, in active as well as inactive condition (Table 1).

Pregnant ovary of *Rousettus leschenaulti* showing the degenerating follicular cells showing low protein staining and the degenerating interstitial cells showing light protein staining (Fig4).

Table 1 : Histochemical Observations in Interstitial cells in *Rousettus leschenaulti*

Ovary Phase	Immature	Inactive Phase	Active Phase	Pregnant
Protein	+	++	+++	+

+ Low/Poor; ++ Moderate; +++ High/Intense

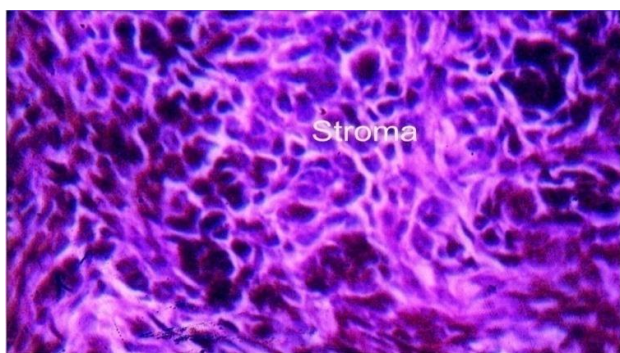


Figure 1

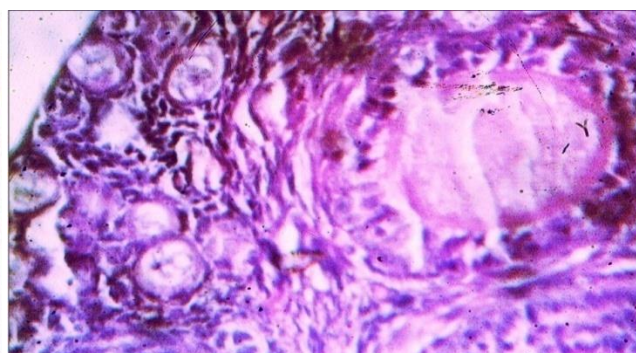


Figure 2

Figure 1 : In the immature ovary of *Rousettus leschenaulti* intensity of protein is less. The developing primordial follicles stain moderately but stromal area stain less with Protein (X100).

Figure 2: Inactive ovary of *Rousettus leschenaulti* showing a moderate to high activity of protein in interstitial cells and in most of the ovarian components. The germinal epithelium cells and the stromal region was moderately stained (X400).IC- Interstitial Cells



Figure 3a

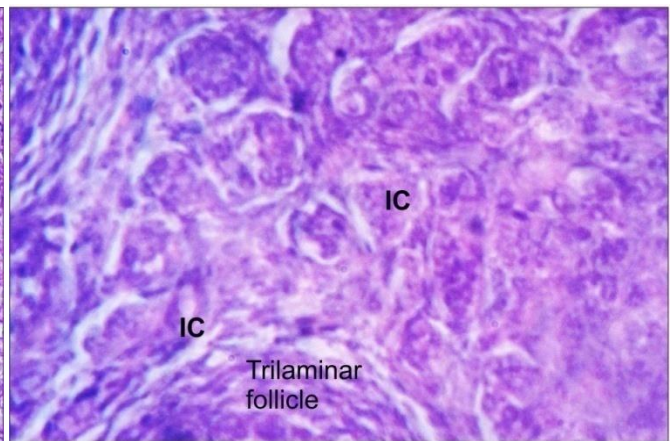


Figure 3b

Figure 3a : Active ovary of *Rousettus leschenaulti* showing the protein staining visualizing in various follicle types, theca externa and interna, interstitial tissue and stroma. The interstitial cell bundles in the stromal area showing high protein staining (X100). TF - Theca Folliculi, IC - Interstitial Cells, CT - Connective Tissues, AF - Atretic Follicle

Figure 3b: Active ovary of *Rousettus leschenaulti* showing the interstitial cell bundles in the stromal area showing high protein staining, thecal area of follicle showing low protein reactivity (X400).

IC -Interstitial Cells

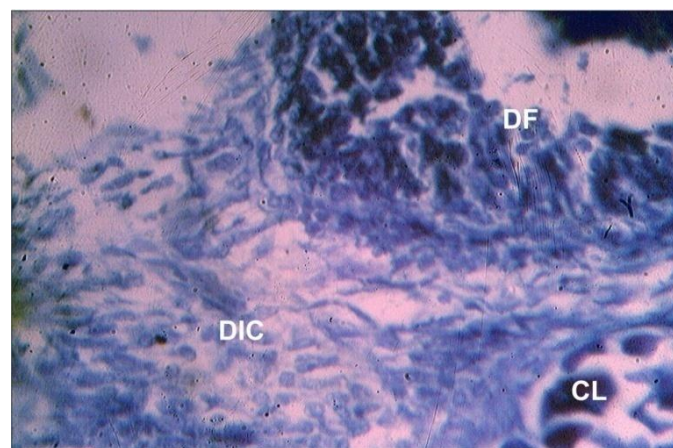


Figure 4 : Pregnant ovary of *Rousettus leschenaulti* showing the degenerating follicular cells showing low protein staining and the degenerating interstitial cells showing light protein staining (X400).

DIC - Degenerating Interstitial Cells, DF - Degenerating Follicles, CL - Corpus luteum

Histochemical observations of Protein in interstitial cells in the ovary of *Megaderma lyra lyra*

The Immature ovary of *Megaderma lyra lyra* showing various developing primordial follicles and the ovarian stroma sparsely distributed between the follicles. There are sparsely distributed clusters of interstitial cells are seen in the stromal region between the follicles showing poor to moderate protein (Fig 5).

It is observed that the protein staining moderate in the inactive ovaries of *Megaderma lyra lyra*. Moderate protein concentration showing in the epithelial, stromal

and thecal areas In inactive condition of ovary, protein staining in the interstitial cells showed moderate. Inactive ovary showed a positive activity of protein in interstitial cells and in most of the ovarian components (Fig 6a and 6b).

In the active ovaries protein staining showed high. It means that when the ovary remains active, It is more secretory than that of inactive ovary. In the active ovary the amount of proteins increases (Fig 7a and 7b).

The distribution of mercury bromophenol positive proteins in the interstitial cells in the ovaries bat

Megaderma lyra lyra and observed in active as well as inactive condition. in the active condition of ovary, protein staining showed high. Ovaries of Active and inactive Bats stained well in mercury bromophenol blue. The cytoplasm of germinal epithelium cells and the stromal region was strongly stained. The primary, secondary, Graffian follicles and zona pellucida exhibited a strong stain. The theca folliculi showed a moderate reaction. The Active ovary of *Megaderma lyra lyra* showing that Protein is significantly present in the

follicular granulosa cells as well as interstitial cells. Protein also showed positive reaction with stromal tissues and blood vessels. Large numbers of Interstitial cell bundles show strong protein reactivity (Table2).

The pregnant ovary of *Megaderma lyra lyra* showing large part of extrovert corpus luteum showing low reactivity with protein, degenerating bundles of Interstitial cells and follicles showing low to moderate protein reactivity (Fig 8).

Table-2 : Histochemical Observations of Interstitial cells in *Megaderma lyra lyra*

Ovary Phase	Immature	Inactive Phase	Active Phase	Pregnant
Protein	+	++	+++	++

+ Low/Poor; ++ Moderate; +++ High/Intense

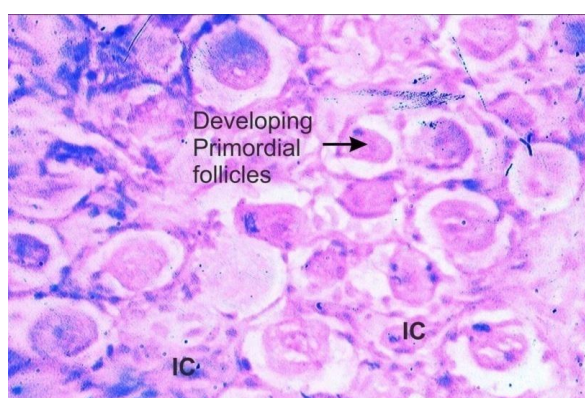


Figure 5: The Immature ovary of *Megaderma lyra lyra* showing various developing primordial follicles and the ovarian stroma sparsely distributed between the follicles. It is made up of connective tissue. There are clusters of interstitial cells are seen in the stromal region between the follicles showing poor to moderate protein. (X200).

IC - Interstitial Cells

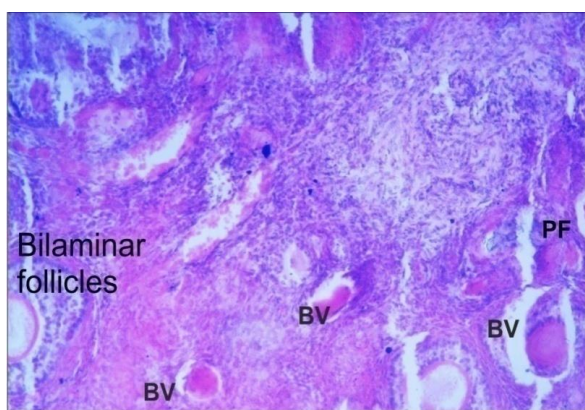


Figure 6a

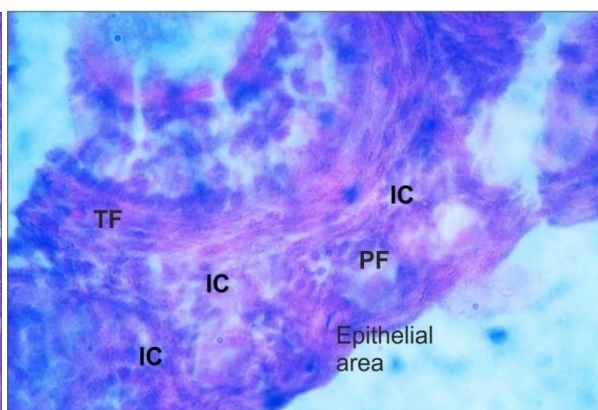


Figure 6b

Figure 6a: The inactive ovaries of *Megaderma lyra lyra* showing the moderate protein staining. More protein concentration showing in the stromal and thecal areas (X100). BV - Blood Vessel, PF - Primordial Follicle

Figure 6b: The inactive ovaries of *Megaderma lyra lyra* showing the interstitial cells with moderate protein staining. Moderate protein concentration seen in the epithelial and thecal areas (X400).

PF - Primordial Follicle, TF - Theca Folliculi, IC - Interstitial Cells

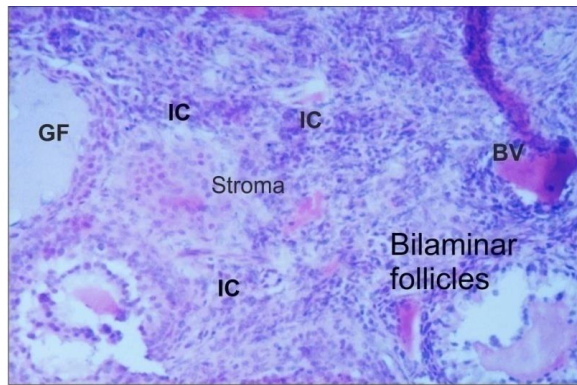


Figure 7a

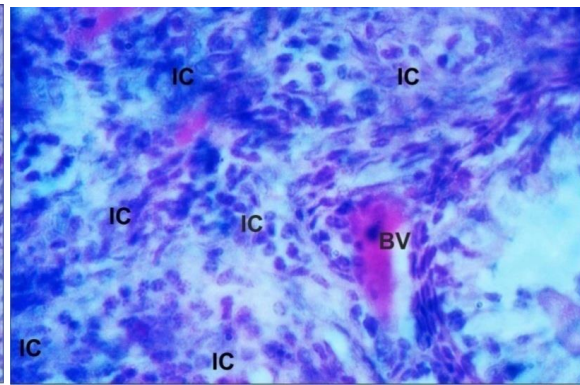


Figure 7b

Figure 7a: The Active ovary of *Megaderma lyra lyra* showing high protein staining in the Interstitial cell bundles distributed in stromal and thecal areas. Moderate protein concentration showing in the granulosa cells and low to moderate in thecal areas (X100). **IC:** Interstitial Cells, **AF:** Atretic Follicle, **BV:** Blood Vessel, **GF:** Graafian Follicle

Figure 7b: The Active ovary of *Megaderma lyra lyra* showing that Protein is significantly present in the follicular granulosa cells as well as interstitial cells. Protein also showed positive reaction with stromal tissues and blood vessels. Large numbers of Interstitial cell bundles show strong protein reactivity (X400).

IC - Interstitial Cells, BV - Blood Vessel

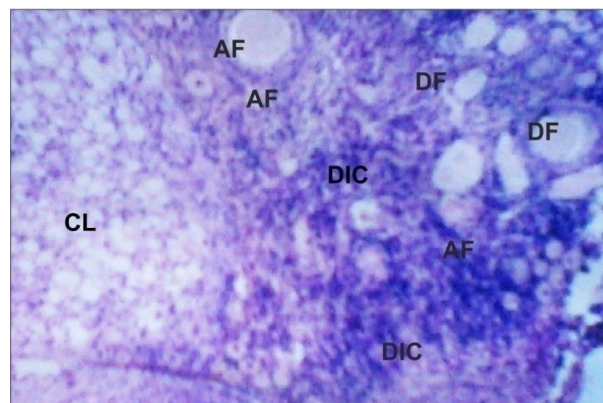


Figure 8: The pregnant ovary of *Megaderma lyra lyra* showing large part of extort carpus luteum showing low reactivity with protein, degenerating bundles of Interstitial cells and follicles showing low to moderate protein reactivity. (X100). **DIC:** Degenerating Interstitial Cells, **DF:** Degenerating Follicles, **CL:** Carpus luteum, **AF:** Atretic Follicle

Histochemical observations of protein in the interstitial cells in the ovary of *Hipposideros speoris*

The protein staining visualized in granulosa and various follicle types, theca externa and interna, interstitial tissue and stroma. This ranges from moderate to high. The granulosa cells of unilaminar and bilaminar follicles show light protein staining. The various preantral follicles show weak staining for proteins.

The immature ovary of *Hipposideros speoris* showing intensity of glycogen is less. The developing primordial follicles stain moderately but stromal area stain weak with protein. No Interstitial cells identified from stromal area (Fig 9).

The inactive ovaries of *Hipposideros speoris* showing the moderate protein staining in the interstitial cells in the epithelial and stromal region. Moderate protein concentration showing in the stromal and peripheral areas, primordial follicles showing low protein reactivity (Fig 10a and 10b).

It is observed that the protein staining moderate in the inactive ovaries of *Hipposideros speoris*. In the active ovaries protein staining show also moderate. It means that in the *Hipposideros* ovary the Interstitial cells becomes secretory in inactive as well as active ovary. In the active ovary the amount of protein increases (Table 3).

The distribution of mercury bromophenol positive proteins in the interstitial cells in the ovaries of *Hipposideros speoris*.

Ovaries of Active and inactive Bats stained well in mercury bromophenol blue Inactive ovary showed a positive activity of protein in interstitial cells and in most of the ovarian components.

The ovarian interstitial cells showed high reactions with mercury bromophenol blue, in *Hipposideros speoris*, in active condition (Fig 11a and 11b).

The pregnant ovary of *Hipposideros speoris* showing large part of extrovert corpus luteum with low protein staining, degenerating bundles of Interstitial cells and follicles also showing moderate to low protein staining (Fig 12).

Table- 3: Histochemical Observations of Interstitial cells in *Hipposideros speoris*

Ovary Phase	Immature	Inactive Phase	Active Phase	Pregnant
Protein	+	++	+++	++

+ Low/Poor; ++ Moderate; +++ High/Intense

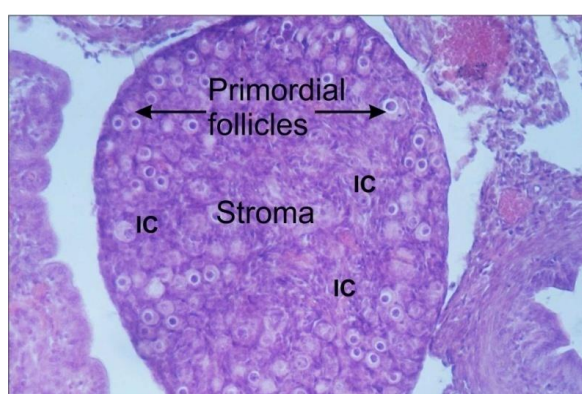


Figure 10a

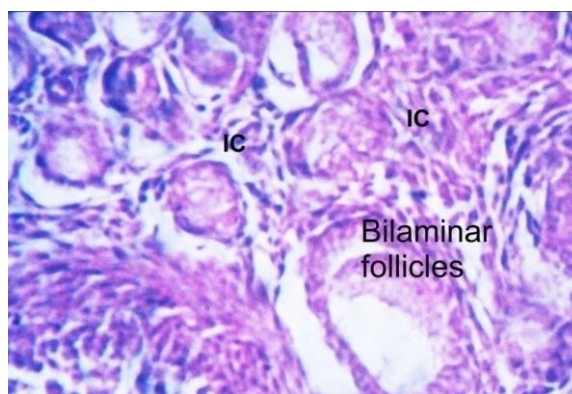


Figure 10b

Figure 10a : The inactive ovaries of *Hipposideros speoris* showing the moderate protein staining in the interstitial cells in the epithelial and stromal region. Moderate protein concentration showing in the stromal and peripheral areas, primordial follicles showing low protein reactivity.(X100). IC - Interstitial Cells

Figure 10b : The inactive ovaries of *Hipposideros speoris* showing the interstitial cells with moderate protein staining. Moderate protein concentration showing in the stromal and low in thecal areas(X100). IC - Interstitial Cells

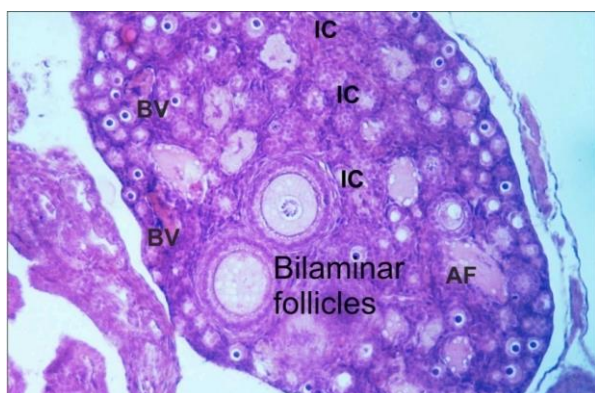


Figure 11a

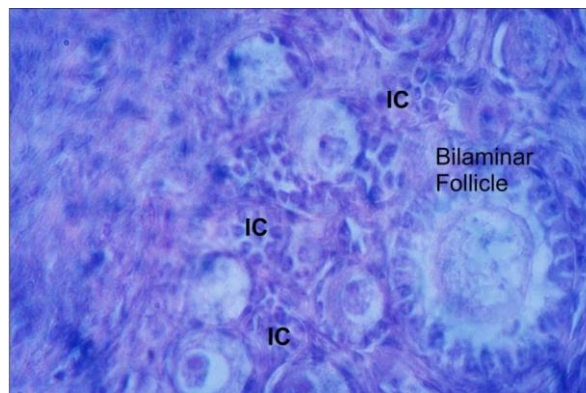


Figure 11b

Figure 11a: The Active ovary of *Hipposideros speoris* showing high protein staining in the Interstitial cell bundles. Moderate protein concentration showing in the granulosa cells, stromal and low in thecal areas(X100).

IC - Interstitial Cells, AF - Atretic Follicle, BV - Blood Vessel

Figure 11b: The Active ovary of *Hipposideros speoris* showing high protein staining in the Interstitial cell bundles. More protein concentration showing in the granulosa cells, stromal and thecal areas (X400).

IC - Interstitial Cells

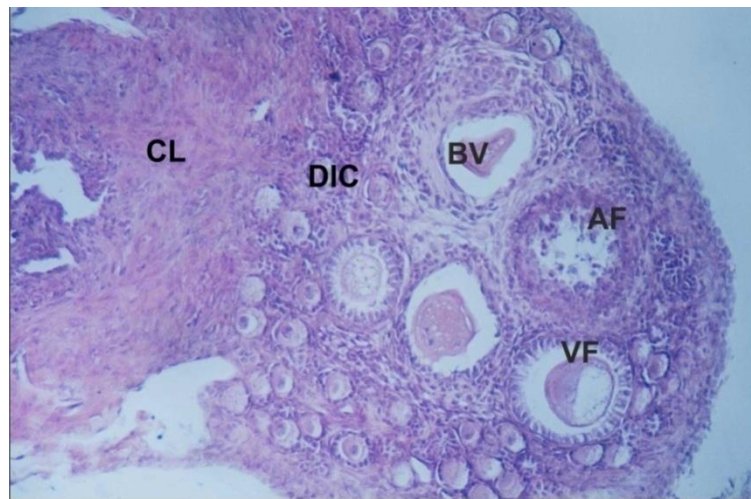


Figure 12: The pregnant ovary of *Hipposideros speoris* showing large part of extrovert carpus luteum with low protein staining, degenerating bundles of Interstitial cells and follicles also showing moderate to low protein staining (X100). DIC - Degenerating Interstitial Cells, DF - Degenerating Follicles, CL - Carpus luteum BV - Blood Vessel, AF - Atretic Follicle

4. DISCUSSION

Mossman and Koering [8] observed the presence of interstitial cells in the stromal region of *Rousettus leschenaulti* in the mature ovary. The interstitial cells are larger than the stromal cells are polygonal in shape. Stroma and interstitial cells supplied with blood vessels. In My Study, in the ovarian stroma of immature *Megaderma lyra lyra* observed numerous cluster of interstitial cells in stromal area. Sastry and Tembhare [9] have designated these cells of thecal origin as the interstitial gland cells in the Mammalian ovary. The second type of interstitial cells in the bat ovary was in the form of carls of smam epithelial cells which were irregularly distributed among the first type of interstitial cells.

Chanda et al. [10] and Krishna A [11] Found that Increase ovarian protein synthesis and secretion during the recrudescence and the preovulatory periods, coinciding with two peaks of follicular development and steroidogenesis. The ovaries synthesized protein at a low rate during quiescence and the late phase of delayed ovulation. Braden [12], Hillard et al. [13], Keyes and Nalbandov [14], Modak and Kamat [15] demonstrated the presence of a polysaccharide protein complex, a neutral and weakly acidic mucoprotein, in the zona pellucida of rat, cat and rabbit. Clark and Book [16], Erickson et al. [17], McManus [18], Silavin [19] observed glycogen and carbohydrate, protein complexes in the

ovary of white rats during the estrus cycle. They noted that glycoprotein was visible in the germinal epithelium, ovum, zona pellucida, follicular granulosa cells, follicular fluid, cells and macrophages of Corpus luteum, intracellular substances basement membrane and atretic ovum. Glycoprotein in the normally developing ovum, which was the only cell containing an appreciable amount of glycogen

In the present study, Interstitial cells in immature ovary showed low staining. Interstitial cells in inactive ovary showed Moderate staining and active ovary showed Intense staining for Protein. Interstitial cells in pregnant ovary showed moderate to low staining for Protein in *Rousettus leschenaulti*, *Megaderma lyra lyra* and *Hipposideros speoris*.

5. CONCLUSION

Rousettus leschenaulti

The immature ovary of *Rousettus leschenaulti* intensity of protein is less. The developing primordial follicles stain moderately but stromal area stain less with Protein.

Interstitial cells in inactive ovary showed Moderate staining and active ovary showed Intense staining for Protein. Interstitial cells in pregnant ovary showed poor staining for Protein. It means that interstitial cells remain more steroidogenic only in active stage of ovary.

Megaderma lyra lyra

Interstitial cells in immature ovary showed low staining. Interstitial cells in inactive ovary showed Moderate staining and active ovary showed Intense staining for Protein. Interstitial cells in pregnant ovary showed moderate staining for Protein. It means that interstitial cells remain more steroidogenic only in active stage of ovary.

Hipposideros speoris

Stromal area in immature ovary showed low staining. Interstitial cells in inactive ovary showed Moderate staining and active ovary showed Intense staining for Protein. Interstitial cells in pregnant ovary showed moderate staining for Protein. It means that interstitial cells remain more steroidogenic only in active stage of ovary.

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