

Original Research Article

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Histoarchitecture and Enzyme Profile Study in Interstitial Glands of Non Pregnant, Pregnant and Lactating Indian Leaf-Nosed Bat *Hipposideros speoris* (Schneider)

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ABSTRACT

This study aimed to investigate the relation between interstitial glands, epithelial cords and pregnancy. The morphological and endocrine aspects of the ovarian interstitial glands of adult female bats were investigated to establish the probable function and the biological significance of this compartment in bats. Pregnant and non-pregnant adult female bats were used for the study. The females were classified according to their reproductive stages in inactive, active, pregnant, and post-lactating. The histological and histochemical features of the interstitial glands were studied. Interstitial glands showed variation in number and morphology during different stages of pregnancy. The cholesterol and its esters were present in non-pregnant females and were scarce in pregnant animals. Histochemical study of enzyme like glucose-6-phosphate dehydrogenase (G-6-PDH), 3 β -hydroxysteroid dehydrogenase (3 β -HSDH), succinate dehydrogenase (SDH) and lipids showed variation at different stages of pregnancy. Our results suggest that the interstitial glands may be storage of precursor substances for the steroidogenesis. These precursors are probably used when the endocrine requirements are high, that is during the pregnancy. Thus this compartment may contribute to the normal gestation of bats. However, the relation between the interstitial cells epithelial cords and the pregnancy is complex, and further studies are needed to clearly establish it.

Keywords

Interstitial glands,
Ovary, Pregnancy

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Introduction

In the order chiroptera, the patterns and strategies reproductive are diverse and consist of the most varied among mammals Peracchi *et al.*, (2006). Four types of reproductive patterns are described for bats seasonal monoestry, seasonal polyestry, non-seasonal

polyestry and bimodal seasonal polyestry (Fleming *et al.*, 1972, Zortea 2003). The micro-chiropteran bat *Hipposideros speoris* was monoestrun bat and that feed on fruits and insect. Ovaries of placental mammals exhibit diversity of structure and function, although the pivotal role of producing ova and steroid hormones is common to all species

chiropterans display variation of ovarian structure and functional adaptations that have few parallels. The interstitial glands of the ovary are not so well defined as those in the testis. *Hipposideros speoris* (order: chiroptera, suborder Microchiroptera and the family Rhinolophidae) is a seasonal breeder, monoestrous and monovular and show sinistral dominance. The mammalian ovary is a complex dynamic structure depicting conspicuous morphological, histochemical, biochemical and molecular changes involving follicles corpora lutea and interstitial gland cells during various reproductive states (Sastry and Pillai 2005, Dorlikar *et al.*, 2013 and Godoy *et al.*, 2014). Of the four major steroidogenically important component of the mammalian ovary viz-developing follicles, atretic follicles, corpora lutea and interstitial gland tissue (Igt), Igt are the most controversial and neglected component of the ovary and least studied. Three types of interstitial gland cells were observed in the ovaries of the bat *Hipposideros speoris* thecal, stromal and epithelial cords all of which exhibit cyclical variation histologically and histochemically in relation with reproductive cycle (Parrott and Skinner 2000, Sastry *et al.*, 2005, 2008, 2010; Singh *et al.*, 2005, Gill *et al.*, 2007 and Trivedi and Lall 2007). We choose to study histochemical localization of SDH, G-6-PDH, 3 β -HSDH and lipids to examine the site and changing pattern of its activity in ovaries of *Hipposideros speoris* during non-pregnant and pregnant states. The purpose of the present study provides a brief account on interstitial gland cells and its role in steroidogenesis during different stages of reproduction.

Materials and Methods

Collection of animals and histology :- All experiments were conducted in accordance with the principles and procedures approved by the Departmental research committee,

RTM University Nagpur, Maharashtra, India. More than 20 specimens of *Hipposideros speoris* were collected once in a calendar month with the help of mist net from natural population inhabiting abandoned mines in Khapa (20°92'N, 78°95'E) Nagpur, Maharashtra throughout the reproductive cycle. These species are found to inhabit in cold and humid places preferably dark. All the bats from the same colony do not show same pattern of reproductive behavior because there exists an asynchrony in reproductive cycle among different females of the same colony. For histological studies the ovaries were fixed quickly in Bouin's fixative, dehydrated in ethanol and embedded in paraffin wax. The sections were cut at 5 μ m, stained with haematoxylin and eosin.

Histochemical detection of lipids

Ovaries fixed in formol-calcium were cut on freezing microtome at - 20°C and were stained by Chiffle and Putt method (Lillie and Fullmer, 1976). These cryocut sections (10 μ m) thick were washed briefly in water and stained with Sudan black B for general lipids. Lipids appeared black or bluish black.

Histochemical detection of enzymes

For histochemical localization of hydroxysteroid dehydrogenase (3 β); glucose-6-phosphatase dehydrogenase (G-6-PDH) and succinic dehydrogenase (SDH) sucrose fixed tissues were cut on cryostat (-20°C) at 10 μ m thicknesses. The incubation medium of 3 β -HSD and G-6-PDH consisted of nitroblue tetrazolium (nitro BT), Nicotinamide adenine dinucleotide (NAD⁺) and pregnenolone dissolved in 2-2 dimethylformamide for 3 β -HSD. For G-6-PD the substrate was glucose-6-phosphate dissolved in 2-2 dimethylformamide. The sections were incubated in substrate media (di-sodium succinate) for SDH. Enzyme product was

visualized by conversion of nitro BT (nitroblue tetrazolium) to tetrazolium granules. Appropriate controls were run in substrate deficient media. The reaction product colour intensity was visually scored as +++= strong, ++= moderate, += low, - = negligible, -- = no reaction (Table-1).

Results and Discussion

The appearance of thecal interstitial gland tissue observed in this study during pregnant and non-pregnant phases as described in other bats, *Rhinopoma microphyllum kinneari* (Trivedi and Lall, 2004; 2007); however, without specifying the reproductive status of the ovary, origin, development and functional status notable to clarify the role of these glands (Singh *et al.*, 2005). The thecal type interstitial cells originate due to hypertrophy or transformation of theca interna of atretic vesicular and multilaminar follicles in *Hipposideros speoris* (Fig. 4 and 10). The thecal Igc are of very transient nature in *Hipposideros speoris* (Fig. 1, 7 and 13) as in few other cases such as women, rhesus monkey, cow and buffalo as they quickly revert to the embryonic stromal. Besides reverting back to the original stromal tissue, some interstitial tissue in *Hipposideros speoris* were also observed to undergo degeneration.

The presence of varying amounts of diffuse lipids and lipid droplets in the interstitial tissue signifies its importance as steroidogenic tissue, as lipid serves as the potential precursor material for steroid biosynthesis (Fig. 16, 19 and 22).

The thecal interstitial glands of *Hipposideros speoris* exhibits a positive reactivity for steroidogenic enzymes 3 β -HSDH (Fig. 16, 19 and 22), SDH (Fig. 34, 37 and 40) and G-6-PDH (Fig. 43, 46 and 49) during inactive and active. Such enzyme activities, indicative of steroid synthesis have been demonstrated in the interstitial gland cells of thecal origin of

some bats (Singh and Krishna, 1994; Singh *et al.*, 2005 Trivedi and Lall, 2004, 2007). The foregoing observations conclude that the process of atresia in the antral and secondary follicles (Fig. 1) in *Hipposideros speoris* appears to be related to the formation of thecal type interstitial gland cells, which maintains ovarian integrity via bio-synthesis, growth rate of follicle, selection of dominant follicle which happened to be one in *Hipposideros speoris*, as the bat is monovular and mono-estrus (Novaldo *et al.*, 2018, Young and McNeilly 2018).

Both morphological and histochemical studies reveal that stromal Igc in *H. speoris* are better equipped for steroidogenesis (Fig. 2, 5 and 11). The histological changes seem to be associated with the histochemical activities of the stromal interstitial cells (Parrott and Skinner 2000). The intensity of the enzymes 3 β -HSD (Fig. 32, 35, 38, 41), G-6-PD (Fig. 55, 56 and 57), SDH (Fig. 44, 47, 50 and 52) and lipid (Fig. 20, 23, 26 and 29) accumulation in stromal interstitial cells in the ovary of *Hipposideros speoris* showed variations during the active, pregnant and lactational phases. Increased lipid accumulation during inactive phase could be due to the sharp decline in androgen production.

All these observations also strongly support the suggestion that the lipid droplets in the Igc are the stores of potential precursor materials which are converted into steroid hormones when the proper gonadotrophic stimulation becomes available. So far, the ovarian stroma (or Igc) has been believed to form mainly androgens (Guraya, 2000) estrogens (Singh and Krishna, 1994; Singh *et al.*, 2005) and progestins (Trivedi and Lall, 2007 and Godoy *et al.*, 2014). However, in the present work the observations on the annual reproductive cycle of the bat, *H. speoris* emphasizes the synthesis of androgens (Fig. 1–50).

Table: 1 The cellular localization and relative activity of different histochemical stains in the interstitial gland (thecal,stromal and epithelial cords) are shown in table-1 correlative to its development only during different phases of reproductive cycle in the ovary.

Chemical substance studied in Igc	Phases of reproductive cycle											
	Recrudescent			Breeding			Pregnancy			Lactation		
	T Igc	S Igc	EC Igc	T Igc	S Igc	EC Igc	T Igc	S Igc	EC Igc	T Igc	S Igc	EC Igc
Lipids (Sudan black B)	+++	+++ +	++	++	++	+++	-	++	++++	-	+++	+++
3β -HSDH	+++	+	++	+++ +	++	++	-	+++ +	++++	-	++	+++
SDH	++	+	++	+++ +	++	++	-	+++ +	++++	-	++	+++
G-6-PDH	++	+	++	+++ +	++	++	-	+++ +	++++	-	++	+++

Note: T Igc : thecal interstitial glands, S Igc: Stromal Interstitial glands and EC Igc: Epithelial cords

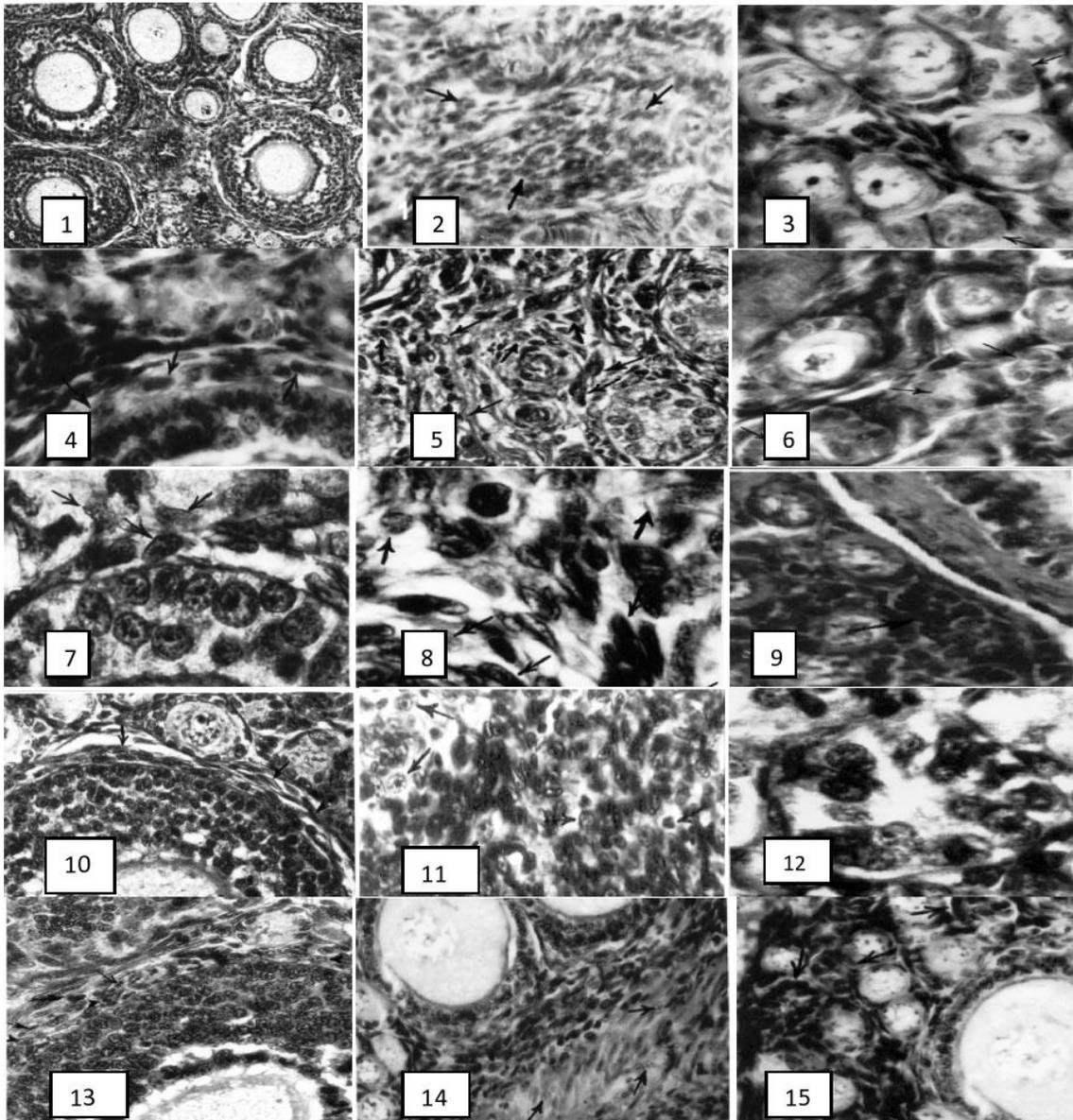


Plate 1: Histological photos showing interstitial glands of reproductive cycle : (Fig. 1-15) Fig.1.Initiation of thecal gland formation inactive ovary. X250. Fig.2. Spindle-shaped stromal cells compactly arranged in the medulla of inactive ovary.X 400. Fig.3 . Reduced but well-defined cords at early active phase X400. Fig.4. Thecal interstitial gland is markedly distinguishable (arrow) from late active phase X 450. Fig.5. The medullary region showing 2-3 small spherical Igc with indistinct nuclei (arrow) from late inactive ovary X 400. Fig.6. Epithelial cords (arrow) distributed individually between follicles at late-active X400. Fig.7. Further increase in the thickness of thecal interstitial band with an increase in the number of cells during active X1000. Fig.8. Late active ovary showing well developed and distinct stromal Igc in the cortical region (arrow) X1000. Fig.9. Part of the ovarian cortex from early pregnancy to show few epithelial cords X400. Fig.10. Part of ovary from (late-active phase) differentiation and development of thecal interstitial gland is of significance (arrow) X450. Fig.11. Note section of ovary displaying the rounded-shaped similarly hypertrophied stromal Igc during mid-pregnancy (arrow) X400. Fig.12. Increase in the number of cords, with simultaneous increase in cell-number (15-16) in each cord during pregnancy. X1000. Fig.13. Thecal interstitial gland cells showing regression (arrow). Fig.14. Lactational ovary showing declined number of stromal Igc (arrow) X400. Fig.15.Recycle of epithelial cords occur with the approach of lactation exhibiting some degenerative EC (arrow)X400.

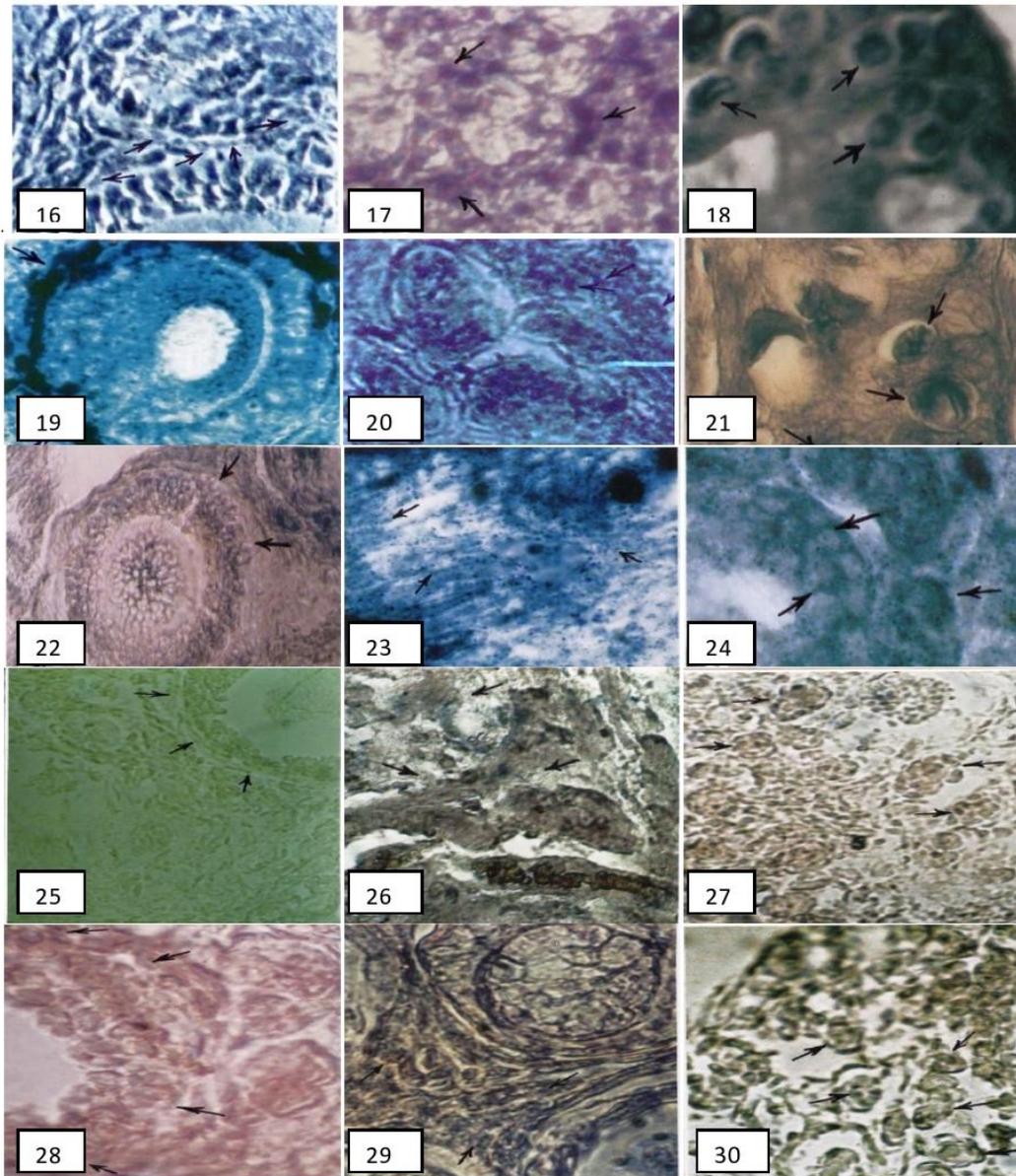


Plate 2: Histochemical photographs showing staining profile in interstitial glands of reproductive cycle:(Fig.16-30): Fig.16. Arrow marks the developing theca having negligible deposition of lipid (early (arrow) inactive phase) X1000. Fig.17. The heavy deposits or droplets of variable but of relatively larger size in the stromal Igc during inactive phase X400. Fig .18.Small-sized inactive epithelial cords from inactive ovary demonstrate for lipids (arrow) X400. Fig.19.Comparabl noticeable moderate lipid activity (arrow) during acive phase in the theca X400. Fig.20. Note lipid droplets during late inactive period which appear strongly positive (arrow) X400. Fig.21.Few cords (arrow) evident in the early active ovary showing lesser amount of lipid droplets.X100. Fig.22.Apparently no reactivity noticed during (late- active) as thecal cells undergo degeneration (arrow) X400. Fig.23.Note lipid activity increase during active.X400. Fig.24.Active phase ovary showing some epithelial cords with lipid accumulation of moderate intensity (arrow) X 100. Fig.25. Thecal gland (arrow), from early active phase exhibiting faint 3 β -HSDH reaction X400. Fig. 26. During advance pregnancy the stromal Igc for sudanophilic reactivity appears negligible (arrow) X400. Fig.27. Epithelial cords from early pregnancy coinciding the implantation of blastocyst demonstrating low intensity lipid droplets along the cell outlines (arrow) X 450.Fig.28. Formazan granular deposition in thecal cells as approaches towards pregnancy. X1000. Fig.29.Lactational ovary stromal Igc are less in number but intensity is slightly decreased (arrow) X400. Fig. 30. Few small epithelial cords from lactating ovary showing reduced lipid droplets along the cellular borders (arrow) X 450.

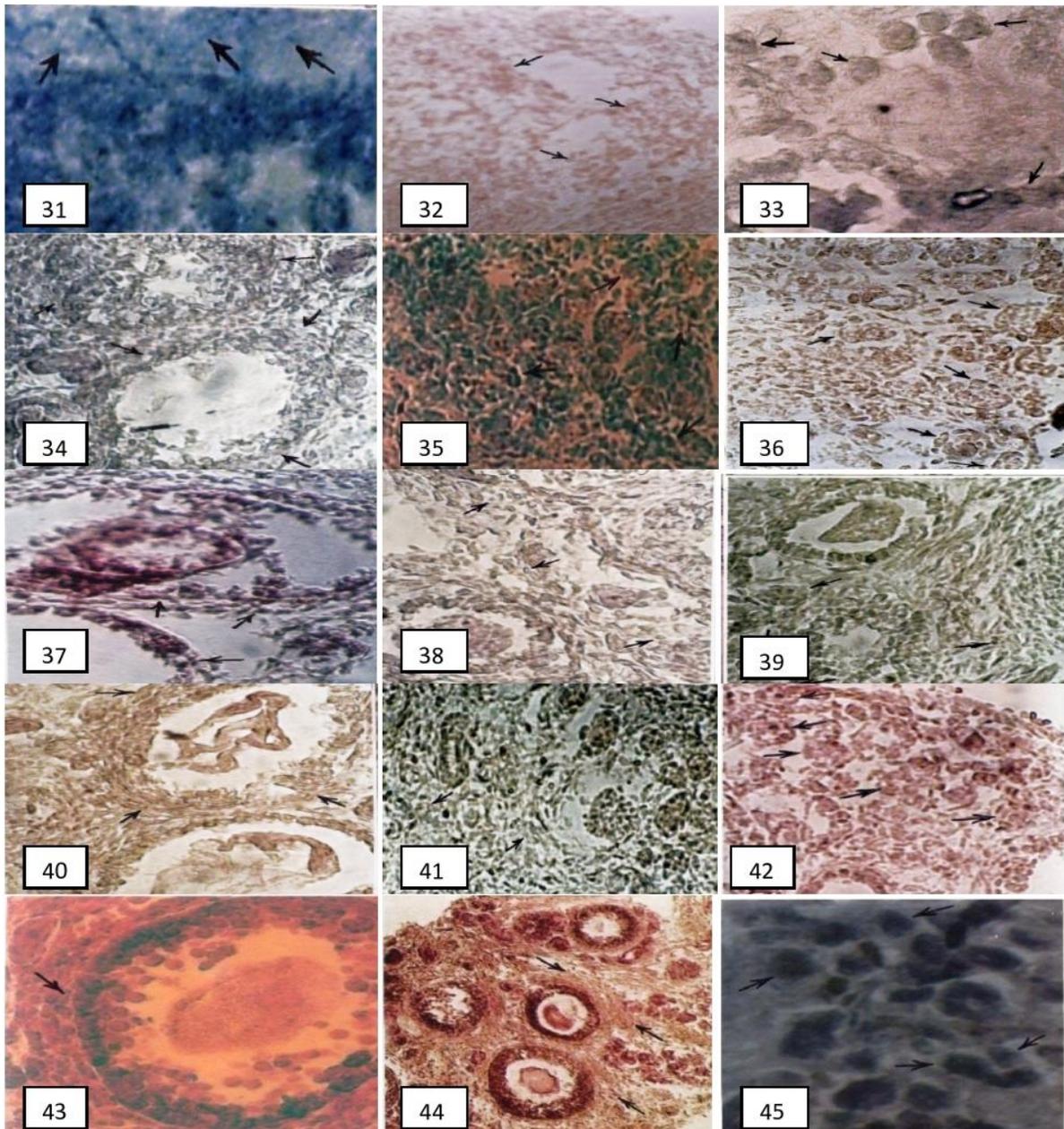


Plate 3: Fig.31.Note during early (late inactive period) thecal gland are undergoing degeneration hence demonstrate low profile for 3β -HSDH (arrow)X400. Fig 32. Early inactive ovary stained for 3β -HSDH reflecting less reactivity in the stromal Igc (arrow) X400. Fig.33.Comparable increase in the 3β -HSDH activity in the epithelial cords from early active ovary (arrow) X400.Fig.34. Slight in SDH activity is detectable in theca during active stage (arrow)X400.Fig.35.Note few granule deposition in stromal Igc during late-inactive (arrow)X400. Fig.36. Reduction in the size of epithelial cords correspond to active period, however, an enhancement of staining profile occurs (arrow)X400. Fig.37.Note thecal interstitial gland exhibiting higher intensity for SDH during November corresponding to active phase (arrow) X400.Fig.38.With the advancement of pregnancy the intensity of 3β -HSDH remained conspicuous and richer (arrow) X1000.Fig.39. Granular deposition of 3β -HSDH in EC.X400. Fig.40. An ovarian section from late active show low SDH activity in theca layer (arrow)X400.Fig.41.Decrease in enzyme activity as approaches towards lactation.X400.Fig.42 Note an abrupt shoot up in the 3β -HSDH reactivity in highly developed epithelial cords (arrow) during mid-pregnancyX250. Fig.43.An antral follicle from early September show developing theca with negligible activity for G-6-PD(arrow)X450.Fig.44.Peripheral region of the late-active depicting positive SDH reactivity in stromal cells(arrow)X400Fig.45. 3β -HSDH reactivity enhances in the lactational ovary with moderate to high activity(arrow) X 100.

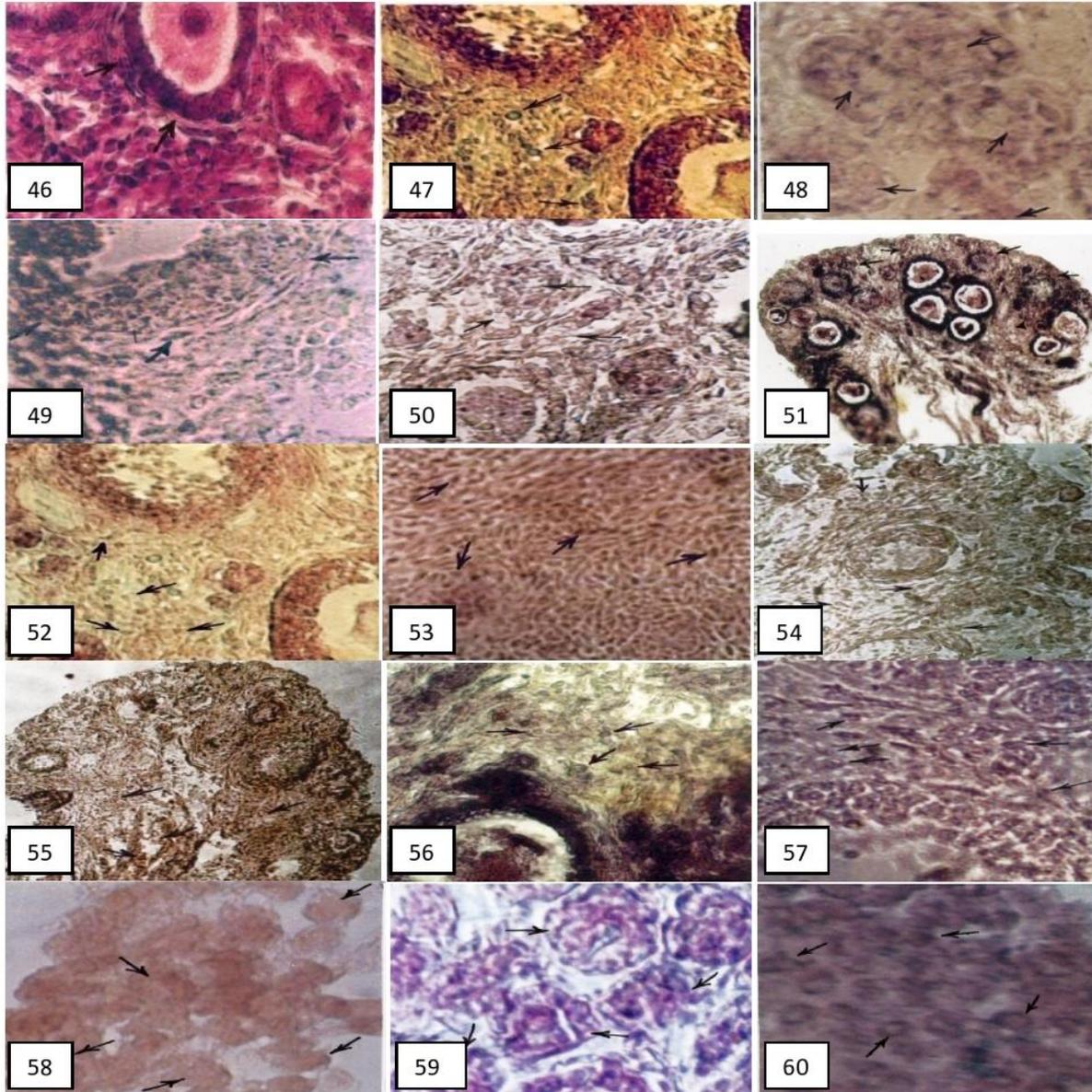


Fig.46.Arrow marks the thecal interstitial gland stained for SDH during active phase.X400. Fig. 47. Enhance in SDH activity as approaches towards active phase.X400. Fig.48 Irregularly distributed small sized cords in early active ovary having some granular deposition for succinate dehydrogenase enzyme (arrow) X400. Fig.49.Regressed thecal Igc (arrow) demonstrating depletion in G-6-PDH reactivity from early active period. X450. Fig. 50. The reaction is concentrated particularly in stromal cells for succinic dehydrogenase in the advance stage pregnant ovary X400. Fig.51. Note greater concentration of SDH granules of succinate dehydrogenase from pregnancy in cords (arrow) X 400. Fig.52. Stromal Igc from lactation exhibiting low to moderate SDH granules (arrow) X400. Fig. 53. Ovary from lactational displaying granular deposition in cords static to previous picture (arrow) X400. Fig 54..Peripheral region of the inactive ovary displaying moderately stained (G-6-PDH) interstitial epithelial cords (arrow) X100. Fig. 55. Ovary at active phase stained for G-6-PDH. Note low to moderate stained stromal Igc in the medullary region (arrow) X100.Fig. 56. G-6-PDH activity in the stromal Igc is at spurt during advanced pregnancy (arrow) X1000. Fig. 57. High power view from lactational ovary displaying depletion of the formazan granules in stromal Igc (arrow) X1000.

The present study also emphasizes that cyclic changes in the interstitial epithelial cords (EC) are related to the reproductive cycle of *Hipposiders speoris*. No one can noticed this type of interstitial gland cells earlier in bats (Gill 2007 and Trivedi and lall 2007). We observed these Igc cells first in *Hipposiders speoris*.

During mid-pregnancy, they were observed to be highly hypertrophied, closely clustered in the cortical portion of the ovary (Fig. 9), each with 20-30 hypertrophied cells in each cord. Early inactive phase the chords are small and inactive and there was a sudden burst in SDH, 3 β -HSD and G-6-PDH staining profile.

Through the activity of SDH is used as a criterion of luteal function, its activity is correlated with presumed sites of hormone production and places of cellular proliferation (Sastry and Tembhare 2008, 2009, Sastry *et al.*, 2010) However G-6-PDH activity was at more elevated levels during advanced pregnancy as evident from the occurrence of density populated, highly hypertrophied zones in the cortex (Gill *et al.*, 2007, Dorlikar *et al.*, 2013, Zahra and Farangis 2015, Nivaldo *et al.*, 2018, Sastry and Pillai 2005, 2008).

Thus we suggest that this compartment may be a storage precursors for the steroidogenesis and then, the precursors are principally used during pregnancy by other ovarian compartments (e.g.corpus luteum) when the endocrine requirements are higher.

The observed histological and histochemical feature of Igc suggest steroidogenic activity, thus these cells may contribute to the total endocrine production synthesized by the ovary. Then the Igc of *Hipposiders speoris* may play a role as a source of steroid precursors during pregnancy and probable further in its maintenance. However, further studies are necessary to conform their roles.

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