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Ultrastructural organization of the corpus luteum of the Indian Emballonurid Bat, *Taphozous longimanus* (Hardwicke)

A.A. Nerkar and M.M. Gadegone

Cell Biology Section, Department of Zoology, Institute of Science, Nagpur – 440001, India

SUMMARY

The paper describes the ultrastructural organization of the corpus luteum of the emballonurid bat, *Taphozous longimanus* (Hardwicke). This bat is continuously asychronous breeder, exhibiting aseasonal polyoestry. During the early part of gestation, the luteal cells are compactly arranged with clearly demarcated boundaries, and show only moderate hypertrophy. Ultrastructural study of luteal cells during early pregnancy reveals the presence of irregularly shaped euchromatic nucleus, rod-shaped mitochondria with tubular or vesicular cristae, numerous lipid droplets and profiles of smooth/agranular endoplasmic reticulum in the form of short tubules or vesicles. The close association of mitochondria with lipid droplets and agranular endoplasmic reticulum in the luteal cells suggests that these cells are actively involved in steroidogenesis. During mid-pregnancy, the luteal cells are hypertrophied and are highly vacuolated. Large number of hypertrophied mitochondria, increased profiles of tubular cisternae of smooth endoplasmic reticulum and abundant lipid droplets in the cytoplasm of luteal cells during mid-pregnancy indicate an increase in the steroidogenic activity.

Key words: Bat, corpus luteum, luteal cells, progesterone, pregnancy

INTRODUCTION

Although Chiroptera are the second largest order of mammals, next only to Rodentia, detailed descriptions of reproductive patterns and associated endocrine structures are known for only a few chiropteran species. The insectivorous emballonurid sheath-tailed bats are a geographically widespread family with 13 genera and 47 species (Bates and Harrison, 1997). The reproductive patterns, however, have been worked out in some detail only in the genus *Taphozous* [*T. georgianus* in Australia (Kitchener, 1973), *T. melanopogon*, (Sapkal and Khamare, 1984) and *T. longimanus* (Gopalakrishna, 1955; Krishna and Dominic, 1982a) in India].

The *Taphozous longimanus* at Nagpur, central India, exhibits an aseasonal polyestrus (continuous) breeding pattern (Gopalakrishna, 1955), whereas the same species at Varanasi, India, shows a seasonally dioestrus (two successive breeding) pattern (Krishna and Dominic, 1982a). This suggests geographic variation in the reproductive pattern of *T. longimanus*. In this species of bat, the female becomes pregnant more than once in a year, with pregnancies following in a quick succession, and there is a physiological alternation of the two sides of genitalia in successive pregnancies. During pregnancy, the ovary of the non-pregnant horn does not appear to remain quiescent but shows all stages of folliculogenesis (Gopalakrishna, 1955). Gopalakrishna and Badwaik (1988) examined the ovaries of several species of bats during different stages of pregnancy and concluded that there were considerable differences in the rate of growth, mode of development, the definitive structure, the duration of existence and the manner of regression of the corpus luteum in different species of bats.

The histological changes in the corpus luteum of Indian bats during pregnancy have been described for *Scotophilus heathi* (Krishna and Dominic, 1988), *Cynopterus sphnix* (Krishna and Dominic, 1983), *Taphozous longimanus* (Gopalakrishna, 1955) and *Rousettus leschenaulti* (Gopalakrishna et al., 1986). The ultrastructure of corpus luteum of bat has been studied in *Macrotus californicus* (Bleier, 1975; Crichton et al., 1990), *Miniopterus schreibersii* (Crichton et al., 1989) and *Miniopterus schreibersii fuliginosus* (Uchida et al., 1984). No information is available on the ultrastructure of corpus luteum of the Indian bats. Hence, the present work was undertaken to study the ultrastructural changes in the luteal cells during pregnancy of *Taphozous longimanus.*

Correspondence to be addressed to: Dr. Archana A. Nerkar email : archana_nerkar1@yahoo.co.in

MATERIALS AND METHODS

Taphozous longimanus (Hardwicke) specimens were collected from Nagpur throughout the year representing different reproductive states. The specimens were brought to the laboratory alive. Mature females were separated from immature females after observing the mammary glands and the pelvic dugs.

For histological examination, the ovaries from pregnant bats were fixed in alcoholic Bouin's fluid. The tissues were dehydrated through graded series of ethanol, cleared in xylene, embedded in paraffin wax and sectioned at 5-6μm thickness in a Leica 2417 microtome (Leica, Jena, Germany). The sections were stained with Ehrlich's haematoxylin and eosin for microscopic observations (Pearse, 1968).

For electron microscopic studies, the ovaries from pregnant bats were fixed in fresh ice-cold 3% glutaraldehyde solution in cacodylate buffer for two hours. The tissues were washed in buffer and post-fixed for one to two hours in 1% osmium tetroxide. The tissues were dehydrated in graded series of alcohol, followed by propylene oxide, and embedded in araldite, which was be polymerized at 60^0C. Ultrathin sections of selected blocks were cut with glass knife, picked up on copper grids and stained with uranyl acetate and lead citrate. The grids were observed in a JEOL-100s Electron microscope (Japan) at 80KU accelerating voltage and photographed.

RESULTS

In *T. longimanus*, the corpus luteum lies within the confines of the ovary (introvert) and persists till parturition. During early pregnancy, the corpus luteum develops very rapidly and occupies nearly half of the ovary. The luteal cells are considerably hypertrophied and packed closely together, so that there are no intercellular spaces in the corpus luteum. The luteal tissue is subdivided into numerous smaller groups of three to four cells, and each group is separated from the adjacent groups by strands of fibrous tissue and spindle-shaped cells. The luteal tissue is richly vascularized. The cells are polygonal and possess irregularly shaped dense nuclei each with one or two nucleoli. The chromatin is sparse and usually confined to a thin layer on the inner aspect of the nuclear envelope.

The mitochondria are round in shape with tubular or vesicular cristae. The mitochondrial matrix is dense and intra-mitochondrial granules are occasionally observed. The Golgi complex is poorly developed. Smooth endoplasmic reticulum (SER) is found as a series of short tubules or vesicles dispersed throughout the cytoplasm. The luteal cells exhibit conspicuous intracellular variation with regard to the number, size and electron opacity of the lipid droplets. The lipid droplets are numerous and irregular in size containing an electron lucent material. In addition, the luteal cells also contain lysosomes. A few cisternae of rough endoplasmic reticulum (RER) with moderate abundance of ribosomes are present in the cytoplasmic matrix. Free ribosomes are seen scattered in the cytoplasmic matrix (Figs.1, 2).

During mid-pregnancy, the corpus luteum enlarges still further and occupies almost the entire ovary. The luteal cells are compactly arranged and are polygonal in shape. The nucleus is round to oval in shape with one or two nucleoli present near the nuclear envelope. The heterochromatin is in the form of chromatin clumps distributed throughout the nucleoplasm but there is a thin aggregation inner to the nuclear envelope.

The hypertrophied mitochondria, round to oval in shape with tubular and lamellar cristae, are distributed throughout the cytoplasm. But in some mitochondria collapsed cristae are observed. The highly limited RER, consisting of short tubular cisternae, are observed at one or two places in the cytoplasm. The SER is the predominant organelle in the luteal cells during this period. They are in the form of short tubules and vesicles and dispersed throughout the cytoplasm. The Golgi complex is indistinct. Groups of round to oval dense lipid droplets are observed in the cytoplasm. These droplets are in close association with the SER and mitochondria.

Two types of luteal cells, on the basis of electron density of the lipid droplets, are observed during mid-pregnancy. In one cell type, lipid droplets are less osmiphilic and in other cell type, the lipid droplets are highly electron dense. Lysosomes are observed (Figs. 3, 4).

As pregnancy advances, there is a decrease in the diameter of the corpus luteum. The luteal cells appear shrunken and luteolysis has been initiated. The cytoplasm is highly vacuolated.

DISCUSSION

The histological changes observed in the corpus luteum of *T. longimanus* are similar to that described in the corpus luteum of *Scotophilus heathi* (Krishna and Dominic, 1988), *Cynopterus sphnix* (Krishna and Dominic, 1983) and *Rousettus leschenaulti* (Gopalakrishna e .al.,

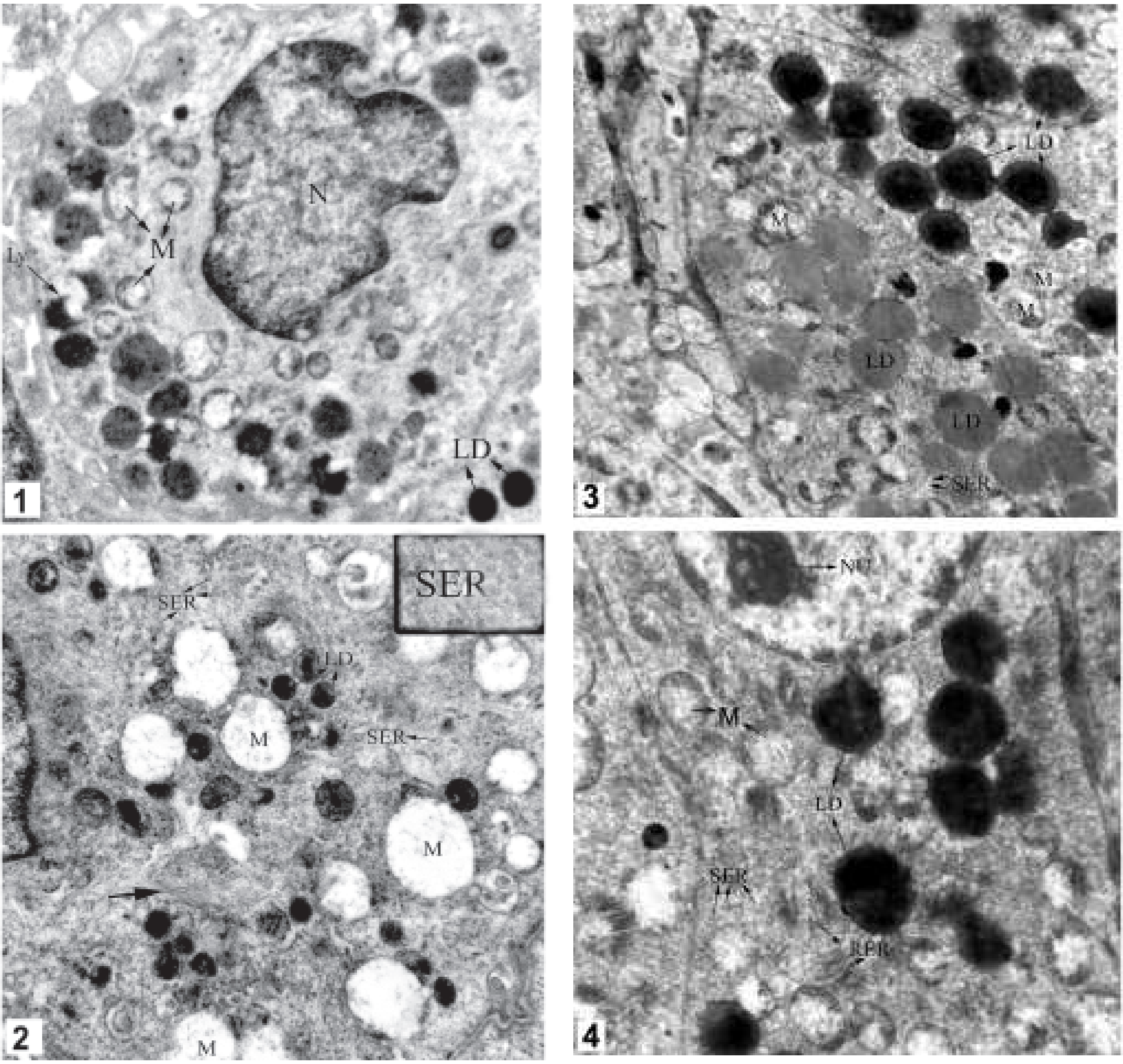

Fig. 1. Electron micrograph of luteal cell during early pregnancy. Note indented nucleus (N), lysosome (Ly), globular mitochondria (M) with vesicular cristae and abundant lipid droplets (LD). X 10000.

Fig. 2. Electron micrograph of luteal cell during early pregnancy showing large number of hypertrophied mitochondria (M) with vesicular cristae, many lipid droplets (LD), rough endoplasmic reticulum (arrow) and vesicular cisternae of SER scattered throughout the cytoplasm. Bundles of microtubules are seen in the cytoplasm (thick arrow). X 15000.

Fig.3. Electron micrograph of luteal cell during mid-pregnancy. Note mitochondria (M) with vesicular or tubular cristae and short tubular or vesicular cisternae of SER. Dark and light osmiphilic lipid droplets are observed in luteal cells. X 10000.

Fig. 4. Electron micrograph of luteal cell during mid-pregnancy showing oval to globular mitochondria with lamellar to vesicular cristae. Dark osmiphilic lipid droplets (LD) of various sizes are observed. Tubular or vesicular cisternae of SER and free ribosomes are seen scattered in the cytoplasm. Short tubular cisternae of RER are also seen scattered in the cytoplasm. X 15000.

1986). The luteal cells of *T. longimanus* during early pregnancy possess irregularly shaped euchromatic nuclei. The mitochondria are rod-shaped with tubular and vesicular cristae, and profiles of SER are in the form of short tubules or vesicles. The lipid droplets are numerous. A close association of mitochondria with lipid droplets and agranular endoplasmic reticulum in luteal cells is regarded as the characteristic features of steroidogenic cells. There is an increase in the steroidogenic activity of luteal cells during mid-pregnancy. The large number of hypertrophied mitochondria, increased profiles of tubular cisternae of SER and large number of lipid droplets in the cytoplasm of luteal cells indicate active synthesis of steroidal hormone during pregnancy. Similar ultrastructural features of luteal cells are reported in other bat species, *Miniopterus sp* (Crichton et al., 1989; Bernard and Bojarki, 1994), *Hipposideros lankadiva* (Seraphim, 2004), *Macrotus californicus* (Crichton et al., 1990) and *Miniopterus schreibersii fuliginosus* (Uchida et al., 1984), during pregnancy.

Christensen and Gillim (1969) reviewed the participation of the SER in steroid biosynthesis, indicating that the enzymes responsible for biosynthesis of cholesterol from acetate and conversion of pregnenolone to progesterone are localized in the SER. Thus, the increased SER complement found in the luteal cells corresponds to the increased plasma progesterone concentration. Enders (1973) suggested that the series of short anastomosing SER tubules found in areas with diverse organelle population represent the best arrangement for progesterone biosynthesis. The presence of lipoprotein, phospholipids and Δ^5 3β-HSD in the corpus luteum throughout the pregnancy of *S. heathi* (Krishna and Dominic, 1988) suggest active secretion of steroid hormones rather than storage of hormone precursors. The high intensity of 3β-HSD and G-6-PD enzymes and lipid concentration in luteal cells during pregnancy of *S. heathi* suggest active steroidogenesis (Singh et al., 2005).

In the present study, there is a close association of mitochondria with SER and lipid droplets in the luteal cells during early pregnancy, and increased abundance of these organelles during mid-pregnancy indicates that the luteal cells of *T. longimanus* might be responsible for progesterone biosynthesis.

In *T. longimanus*, two types of luteal cells, on the basis of nature of lipid droplets cells, are observed in the luteal cells during mid-pregnancy. In one cell type, lipid droplets are less osmiphilic (light) and in other cell type lipid droplets are dark or with high electron density. The light lipid droplets, along with mitochondria and SER, may be involved in active steroidogenesis. The cells with dark lipid droplets along with the other organelles may indicate less steroidogenic activity. However, both the cell types appear to be different states of the same cell type, and the dark cells appear to be a more advanced state than the light cells, and may represent the beginning of luteal regression. In *Minioptreus*, Uchida, et al. (1984) reported two types of cells, light and dark, in the corpus luteum, supporting the present observations. Levine et al. (1979) suggested that the pale lipid droplets may contain relatively greater amount of steroids than the more opaque lipid droplets. The appearance of paler heterogeneous lipid droplets might represent a cholesterol build up during luteal regression.

The SER whorls found in luteal cells of many species have been considered as indicative of cholesterol storage or functional inactivity. These whorls are, therefore, indicative of luteal regression (Bjersing, 1967). This type of SER whorls are not found in the luteal cells of *T. longimanus* (Singh and Krishna, 2002). The accumulation of coarse lipid droplets, consisting of neutral lipids, immediately before and after parturition, indicates the relative inactivity of the corpus luteum and storage of hormone precursor. The occurrence of darkly staining coarse lipid granules in the corpora lutea of post-partum *S. heathi* signifies luteal regression (Krishna and Dominic, 1988). Rarefied and swollen mitochondria and dark lipid droplets are observed in the luteal cells of *Taphozous* during second half of pregnancy. Since mitochondrial enzymes catalyze the rate-limiting step in progesterone synthesis, it is suggested that mitochondrial disruption may be an efficient way to terminate luteal progesterone synthesis.

The luteal cells during mid-pregnancy of *Taphozous* show increased steroid synthesizing capacity as evidenced by the development of steroid producing cell organelles. The persistence of functional corpus luteum in the ovary of *T. longimanus* throughout gestation indicates that the ovary might be the major source of the progesterone necessary for the maintenance of pregnancy in this species.

Singh and Krishna (2002) studied the relationship between ovarian activity and circulating steroid concentration in *T. longimanus* collected from Varanasi, India, where this bat breeds twice a year. Two peaks of progesterone concentrations during the reproductive cycle coincide with the two pregnancies. Interestingly, low progesterone concentration was observed during early phase of the first pregnancy, which might explain the incidence of slow embryonic development in this species of bat. *T. longimanus* is seasonally polyestrus at Nagpur, Central India. It breeds all the year round (Gopalakrishna, 1955). We did not measure the progesterone level in blood plasma during pregnancy. However, the present observations suggest that there is no slow embryonic development during early pregnancy of first pregnancy cycle, and the ultrastructural features of luteal cells during early pregnancy suggest that the luteal cells in the corpus luteum of *T. longimanus* are steroidogenically active and involved in biosynthesis of progesterone, which is necessary for the maintenance of pregnancy.

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