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Light and Ultrastructural Studies on Corpus luteum of bat *Taphozous kachhensis* (Dobson)

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Abstract: The ultrastructure of Corpus luteum and the different cell types in the functional corpus luteum of the bat were studied using the electron microscope. Two types of luteal cell, a large and small, were present in the corpus luteum of this species of bat. The large more rounded luteal cell possessed numerous cell organelles such as mitochondria and electron dense membrane-bound granules, agranular endoplasmic reticulum and granular endoplasmic reticulum which at times appeared as stacks of cisternae. With this lipid droplets were present in the luteal cell cytoplasma while whorled agranular endoplasmic reticulum was absent. Small luteal cell present amongs the large luteal cells with tapering cytoplasmic processes. These cells differed from the large luteal cells in that they possessed fewer mitochondria and electron dense membrane- bound granules. Occasional nuclei of the small luteal cells contained cytoplasmic inclusion bodies.

I. INTRODUCTION

The endocrine role of the ovary is to secret progesterone and estrogen and hormonal regulation of pregnancy. It is well observed in various chiropterans as for as the endocrine role of ovary during pregnancy is concerned. One of the hormones of ovary, progesterone is secreted from the corpus luteum. The corpus luteum is a transient endocrine gland required for successful maintenance of pregnancy.

The corpus luteum is a transient endocrime gland that is specialized for the production of progesterone and that plays a critical role in the establishment and maintenance of pregnancy. The life span of the corpus luteum varies between species and, within a species, can be dramatically altered by events such as mating or pregnancy.

A corpus luteum is Ipsilateral to the reproductive duct carrying the newly ovulated ovum or conceptus is reported in *Haplomycteris fischeri* by Heidman (1989). He observed a few cases of partly extrovert corpus luteum and in most instances the corpus luteum formed a spherical mass that filled 25-90% of the ovary. *Haplomycteris fischeri* exhibits embryonic developmental delay during its reproductive cycle. By the time blastocyst has reached the uterus, the corpus luteum was vascularized. The corpus luteum of early pregnancy and early delay typically had large cell with large nuclei and prominent nucleoli. Corpus luteum of females with embryos in delay or soon after the end of play also contains large lutein cells. It is during late pregnancy when most cells were much smaller and the corpora lutea had and therefore decreased considerably in size. By the last month of gestation, the involution corpora lutea had greatly diminished and is no longer apparent by the time of implantation of the next embryo. Thus in pygmy bats, there were no clear changes in the lutea cells over the delay period of embryonic development

The examination of the ovaries of several species of bats during different stages of pregnancy reveled that there were considerable difference in the rate of growth, mode of development, the definitive structure, the duration of existence and manner of regression of the corpus luteum among the different species of bats (Gopalkrishna and Badwaik, 1988).

Ultrastructure of corpus luteum is studied in *Miniopterus schreibersii* during delayed development by Crichton et.al. (1989).They also reported ultrastructure of corpus luteum during different stages of development.

Ultrastructural studies of corpus luteum by Bleier (1975) showed that the structure of corpus luteum in *Macrotus californicus* is similar to *Artibeus jamaicensis*. He found that the corpus luteum was metabolically more active during nidation and implantation and inactive during diapause. Later it was quiet active from arousal until the termination of pregnancy in *Macrotus californicus*. It may be the low amount of luteotropic harmone (prolactine) that is responsible for a less metabolically active corpus luteum during diapouse (Richardson, 1981).

Luteinizing hormone LH from the anterior pituitary is important for normal development and function of the corpus luteum in most mammals, although growth hormone, prolactin and estradiol also play a role in several species (Niswender, et.al. 2000).

Although the endocrine glands undoubtedly play a major role in regulating sexual cycle of mammals, there is a little information available on structural and functional correlates to reproductive function of the Indian bats.

II. MATERIAL AND METHODS

The specimen of Taphozous *kachhensis* were collected from Ambai Nimbi, about 45 kilometers from Bramhapuri (M.S.). Many collections were made during the breeding season so as to coincide with the time of reproductive cycle and to get an accurate pregnancy record. During the day time, their roosting places were visited and the specimens were netted at random with the help of a butterfly net. These bats are very sluggish in nature after collection they were sexed and only the females were brought to the laboratory. For light microscopy ovary was dissected out and fixed in alcoholic Bouin’s fluid. After fixation for 24 hr tissue were washed with 70% ethanol. For histological observation, the tissues were later dehydrated in various grades of alcohol, cleared in xylol, and embedded in paraffin wax. The tissues were cut at 5 to 6µwith the help of Leica 2417 microtome the sections were stained with haematoxylin and eosin for routine histological examination. For electron microscopy the tissue were fixed in 4% glutaraldehyde in 0.1 M sodium cocodylate buffer (pH 7.2) at 4°C. The tissues were post fixed in cacodylate-buffered 1% osmium tetroxide, dehydrated in a graded series of dilutions of acetone in distilled water and embedded in araldite. Thin sections were stained with a saturated solution of uranyl acetate in 70% filtered methanol, and lead citrate and examined with a Philips HMG 400 electron microscope.

III. OBSERVATIONS

A. Ovary During Early Pregnancy

Specimen collected in the month of January show the early pregnancy. At this stage two ovaries show different histological pictures. The right ovary shows the presence of well developed introvert corpus luteum which appear as a solid ball like structure occupying almost 1/3 part of the ovary (Fig. 1). The luteal cells are compactly arranged and show moderate hypertrophy. Nuclei are distinct and darkly stained with Chromatin clumps. Cytoplasm is granular and vacuolizations are observed in most of the cells. (Fig. 2). Ovary on the left side shows the follicle in all stage of development up to multilaminar stage.

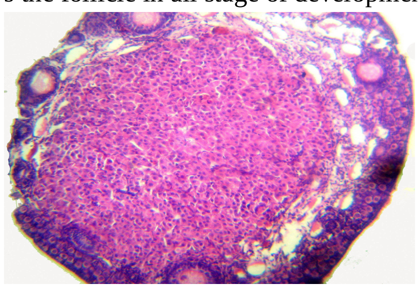
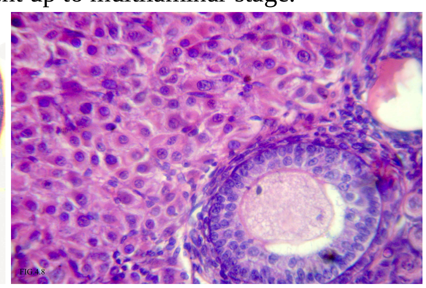

Fig. 1 T. S of the ovary during early pregnancy showing the presence of introvert corpus luteum (CL). X 100

Fig. 2 Magnified view of the corpus luteum showing the presence of luteal cell with granular cytoplasm. Note the stages of mitotic division in the luteal cells. In some cell cytoplasm is vacuolated.X 1000

B. Ovary During Mid-Pregnancy

Specimens collected in the month of March showed mid-pregnancy. During this stage the corpus luteum is fully developed and it occupies the entire ovary except small peripheral region; (Fig. 3) where few follicles are seen.

During this stage corpus luteum reaches its maximum size. In peripheral region some follicles continues to develop up to unilaminar or bilaminar follicles. The corpus luteum consists of hypertrophied, highly vacuolated luteal cells. At this stage nuclei are very distinct, Cytoplasm is deeply stained and exhibit granular appearance (Fig. 4).

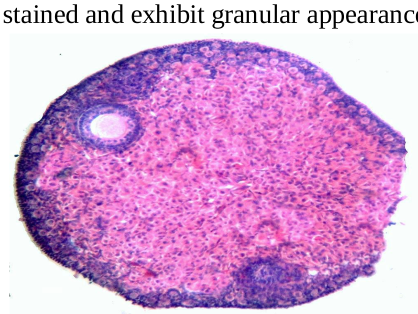
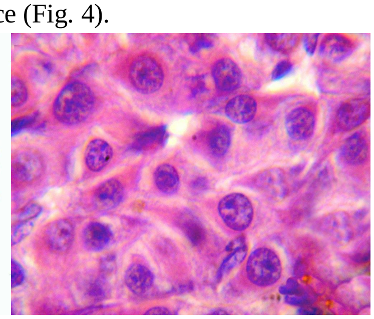

Fig. 3 T.S. of ovary during mid-pregnancy showing the presence of well developed corpus luteum, few primordial follicles, and double layered follicle. X 100

Fig. 4 Enlarged portion of the corpus luteum during mid-pregnancy showing hypertrophied, highly vacuolated luteal cells (LC). Note the presence of spherical nuclei. X 1000

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C. Ovary During Late-Pregnancy

Specimens collected in the month of May show the late pregnancy. During this stage there is progressive decrease in the size of corpus luteum. Many luteal cells are shrunken. Intracellular spaces in the corpus luteum have increased. Small vacuoles are also seen in the cytoplasm of the luteal cells. The process of luteolysis has been initiated in the luteal cells (Fig. 5).

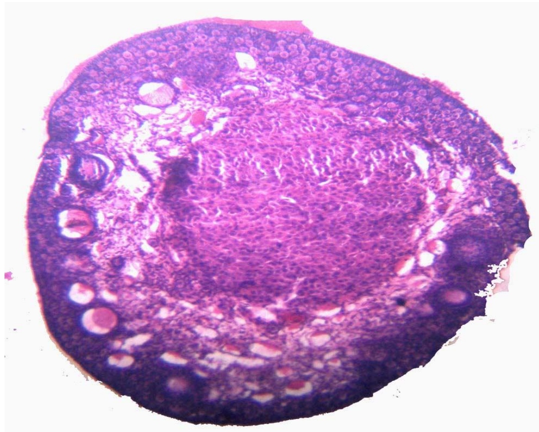

Fig. 5 T.S. of ovary during late pregnancy showing the presence of regressed corpus luteum (CL) X 100

D. Ovary during lactation

After the parturition, the females were in lactating stage in the month of June. The young one is attached to the body of the female. The ovary of the parturated horn contains degenerating corpus luteum in the form of scar like body or the corpus albicans; most of the follicles are in degenerating state leaving only empty follicles. While the ovary of non-parturated horn contains few unilaminar follicles. Primordial cell are still persisted in the peripheral part of ovary. The connective tissue stroma is poorly developed during this stage.

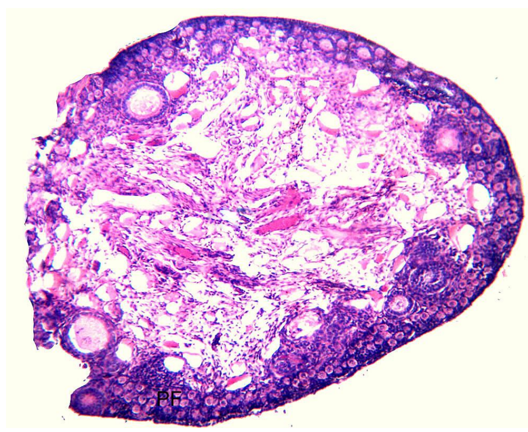

Fig. 6 T.S. of ovary of non-parturated horn during lactation showing some atretic follicles. X 400

IV. ELECTRON MICROSCOPIC STUDY

A. Ovary During Early Pregnancy

During the early pregnancy, the ovary shows well developed introvert corpus luteum. The corpus luteum occupies nearly half of the ovary. The luteal cells shows hypertrophy and are packed together. These luteal cells are divided into smaller group of two to three cells. These groups of cells are separated from each other by a strand of connective tissue. The luteal cells are polygonal in shape and shows irregularly shaped nucleus with one or two nucleoli (Fig 7). Mitochondria are spherical to oval in shape with vesicular cristae. Golgi body is seen and it is juxtanuclear in position. Few lipid droplets are seen in the cytoplasm. Smooth endoplasmic reticulum is found in series of short tubule and dispersed throughout cytoplasm, thus the close association of mitochondria and Smooth endoplasmic reticulum and lipid droplets in the luteal cells is regarded as characteristic structure of steroidogenic organelles. Free ribosomes are seen scattered in cytoplasmic matrix (Fig 8).

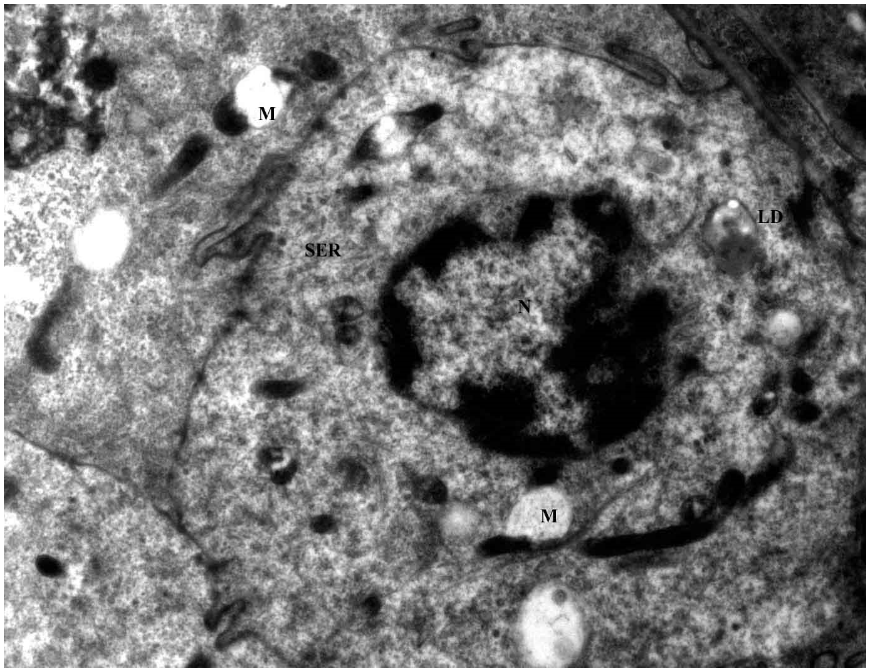

FIG.7 Electronmicrograph of the luteal cell during early pregnancy. Note the presence of indented nucleus (N), mitochondria (M) with collapsed cristae and lipid droplet (LD) x 4000

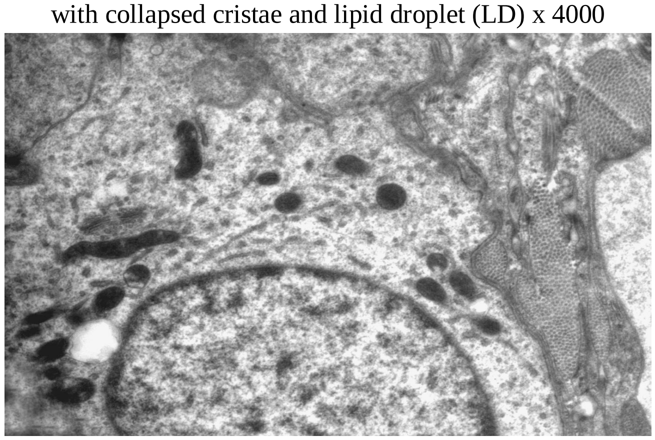

FIG. 8 Electronmicrograph of the luteal cell during early pregnancy. Note the presence of abundant mitochondria (M), rough endoplasmic reticulum (RER), Golgi apparatus (G) and lipid droplet (LD) X4000

B. Ovary During Mid- Pregnancy

During mid pregnancy the corpus luteum has occupied almost the whole part of the ovary the luteal cells are polygonal in shape with spherical to round nucleus. The chromatin clusters are distributed throughout the nucleoplasm (Fig 9 & 10).

Mitochondria are abundant and uniformly scattered in the cell cytoplasm. These are elongated in shape with lamellar cristae (Fig 11) and are close association with Golgi-SER zone (Fig 12). The smooth endoplasmic reticulum is present in large number in the luteal cells. Mostly vesicular profile of smooth endoplasmic reticulum are observed and scattered through the cytoplasm (Fig 13).

Golgi apparatus is well developed and two large Golgi zone are observed in the luteal cell. These are juxtanuclear in position. A large number of small of associated vesicle are observed near the laminar part of Golgi saccules. The maturing face of Golgi is observed occupied by large number of small Secretory vesicle. (Fig 14).

A large number of dense bodies are observed in the cell cytoplasm, some of these contain moderately fine homogenous matrix in which small dark granules are embedded. These bodies are mainly round in shape. Occasionally lipid droplets are seen in the cytoplasm of the luteal cell (Fig 14).

Two type of luteal are observed during mid pregnancy on the basis of electron density. In first type of cell lipid droplet are less osmophilic and in other cell type lipid droplets are with high electron density (Fig 16 & 17). The luteal cell with dark lipid droplets suggest the beginning of luteal regression and those are with light lipid droplets may be involved in active steroid synthesis.

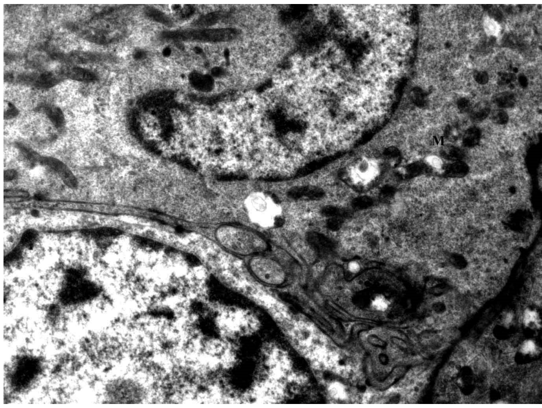
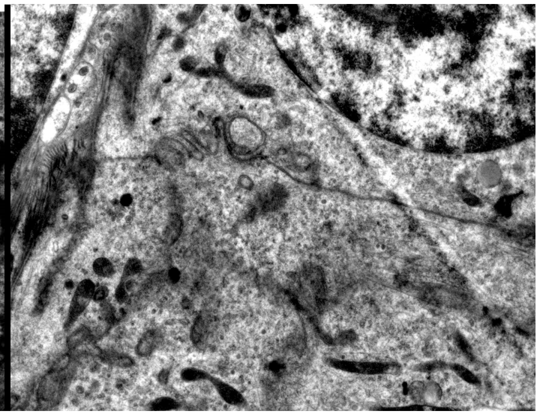
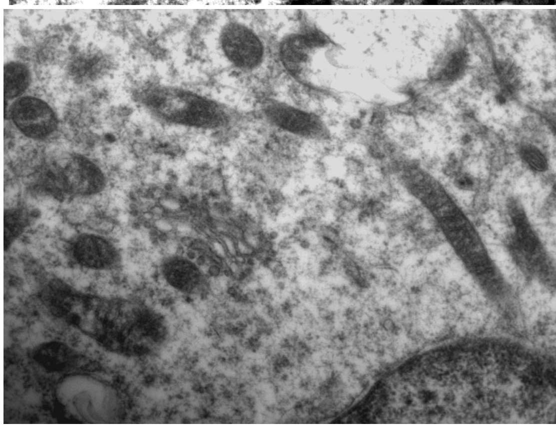
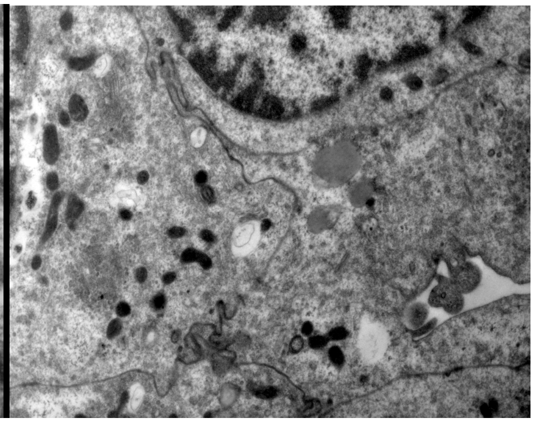
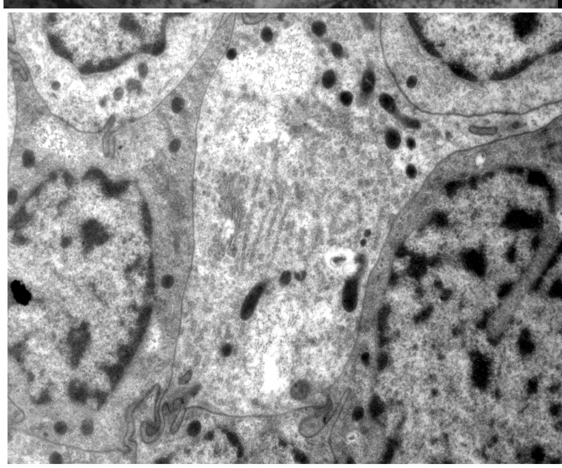
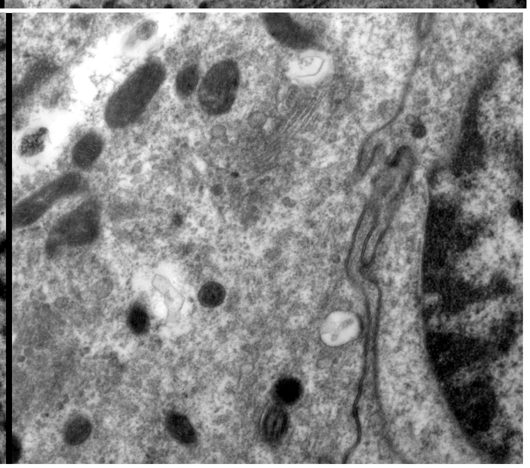

FIG. 9 Electronmicrograph of luteal cells during mid pregnancy. Note the presence of abundant mitochondria (M) with lamellar cristae X 8000

FIG.10 Magnified view of the luteal cells during mid pregnancy. Note the presence of nucleus(N) with prominent nucleolus (NO) large number of mitochondria (M) with tubular cristae, lipid droplets (LD) , tubular cisterna of smooth endoplasmic reticulum (SER) are seen X 6000

FIG. 11 Electronmicrograph of luteal cells during mid pregnancy. Note the presence mitochondria (M) with lamellar cristae and Golgi apparatus (G) with large number of vesicles X 10000

FIG. 12 Electronmicrograph of luteal cells during mid pregnancy. Note the presence of large number of mitochondria (M), lipid droplets (LD) and Golgi apparatus (G) X6000

FIG. 13 Electronmicrograph of luteal cells during mid pregnancy. Note the presence of Rough endoplasmic reticulum (RER) studded with ribosomes. X 4000

FIG. 14 Magnified view of the luteal cell during mid pregnancy. Note the presence of massive Golgi apparatus (G). Note the presence of dense body in the cytoplasm X 8000

V. DISCUSSION

The corpus luteum occupies the entire ovary and the formation of extrovert corpus luteum also is reported in *Megaderma lyra lyra* (Sonwane, 2010) and *Hipposideros speoris* (Gopalkrishna and Bhatiya, 1983).The presence of introvert corpus luteum has been observed in *Taphozous longimanus* (Nerkar, 2007). Penduculate corpus luteum is reported only in *Rhinolopus rouxi* (Ramakrishna *et.al*. 1981) and *Hipposideros lankadiva* (Sapkal and Bhandarkar, 1984; Seraphim 2004). The rapid enlargement of corpus luteum as noticed in *Taphozous kachhensis* is also reported in other Chiropteran species such as *Cynopterus sphinx* (Krishna and Dominic, 1983) and *Taphozous longimanus* (Nerkar (2007). The corpus luteum is a transient endocrine gland required for successful maintenance of pregnancy and one of the ovarian hormone's progesterone is secreted from the corpus luteum. Corpus luteum is reported to persist for several months after parturition in *Rousettus leschenaulti* (Gopalkrishna et. al., 1986) and *Cynopterus sphinx* (Krishna and Dominic, 1983). The corpus luteum is short lived and disappears very early in pregnancy in some bats such as *R. rouxi* (Ramakrishna, 1978), *Pipistrellus mimus mimus* (Krishna, 1985), *Megaderma lyra lyra* (Sonwane, 2010) and *Hipposideros lankadiva* (Seraphim 2004). In contrast, corpus luteum persist till parturition in *Taphozous longimanus* (Nerkar, 2007), *Scotophilus heathi* (Krishna and Dominic, 1988). In most of the mammals corpus luteum is composed of two distinguishable cell types, small size luteal cells and large size luteal cells (Koering, 1974; Paovola, 1977) and is derived from both granulosa and theca layer of ovarian follicle. In Chiroptera, the study of corpus luteum has been reported in *Taphozous longimanus* (Nerkar, 2007), *Hipposideros speoris* (Ramakrishna et. al., 1981), in *Miniopterus schreibersii* (Bernard et. al., 1991), *Megaderma lyra lyra* (Sonwane, 2010).

The ultrastructural observation of the corpus luteum of *Taphozous kachhensis* during early pregnancy confirms the light microscopic structure of the corpus luteum during early pregnancy. Luteal cells during early pregnancy shows the presence of irregularly shaped nuclei with one or two nucleoli. Mitochondria are spherical to oval in shape with vesicular cristae. Golgi body is juxtanuclear in position. Few lipid droplets are also seen in the cytoplasm. The close association of mitochondria with lipid and smooth endoplasmic reticulum in the luteal cells is regarded as the characteristic feature of the steroidogenic cells. There is increase in the synthetic activity of luteal cells during mid pregnancy. A large number of hypertrophied mitochondria, increased profile of smooth endoplasmic reticulum and lipid droplets are observed in the cytoplasm of luteal cells. These organelles are the indicative of steroid synthesis during pregnancy. Similar ultrastructural finding in the luteal cells are reported in other bat species, *Hipposideros lankadiva* (Seraphim, 2004), *Taphozous kachhensis* (Nerkar, 2007). Ultrastructure of corpus luteum is studied in many mammals, rhesus monkey, (Gulyas, 1974); mouse (Thomas and Browning, 1968); Seals (Sinha and Erickson, 1972) and it was concluded that the morphological features of the luteal cells during pregnancy are consistant with those considered to be necessary for steroidogenic activity, and this supports the present observation. The morphological features of the luteal cells in this species are comparable to those described for steroid secreting cells generally, and for luteal cells of other mammals (Fawcett et. al., 1969; Christensen and Gillim, 1969; Enders, 1973). Many features in the luteal cell of the goat were however similar to those reported in the sheep which include presence of a few lipid droplets and absence of concentric whorls of agranular endoplasmic reticulum in the cytoplasm of the large luteal cells (McClellan et. al., 1975). Large concentric whorls of agranular ER on the other hand have been reported during maximal steroid synthesis in the sow (Bjersing, 1967; Goodman et. al., 1968); rabbit (Blanchette, 1966b); bat and ferret (Enders, 1973); hamster (Leavitt et. al., 1973) and dog (Abel et. al., 1975). Other features of the goat corpus luteum which are similar to that of the sheep are the proportion of the two luteal cell populations, and the presence of cytoplasmic inclusions in small luteal cells (O'Shea et. al., 1979). Christensen and Gillim (1969) reviewed the involvement of the SER in steroid biosynthesis indicating that the enzymes responsible for the biosynthesis of cholesterol from acetate and conversion of pregnenolone to progesterone was located in SER. Thus the increased SER complement found in the luteal cells correspond to the increased plasma progesterone concentration. In the present study, there is an association of mitochondria with smooth endoplasmic reticulum and lipid droplets in the luteal cells during early pregnancy and increased complement during mid pregnancy indicate that luteal cells of corpus luteum of *Taphozous kachhensis* are responsible for progesterone biosynthesis. In the female bat, the pattern and levels of increase in progesterone concentration and its decline after the luteal phase is similar to that found in other species of bats. The progesterone concentration remains at basal levels throughout the estrus, as observed by others (Sonwane, 2010; Khadiga *et.al*, 2005). The cyclic pattern of progesterone concentration in plasma observed during present study is in agreement with known changes in corpus luteum function in the species that occur during the estrous cycle. The rapid decline of progesterone in the peripheral plasma of the bat towards the end of the cycle as well as the marked rise in concentration during the time of CL development is a strong evidence for suggesting that its functions can be monitored in peripheral plasma by progesterone determination. The plasma progesterone levels would decrease rapidly with declining CL function is also suggested by other workers (Sonwane, 2010., Imori, 1967). The results of present study suggest that the luteal cells of corpus luteum are steroidogenically active and involved in the synthesis of progesterone required for the maintenance of pregnancy in this species.

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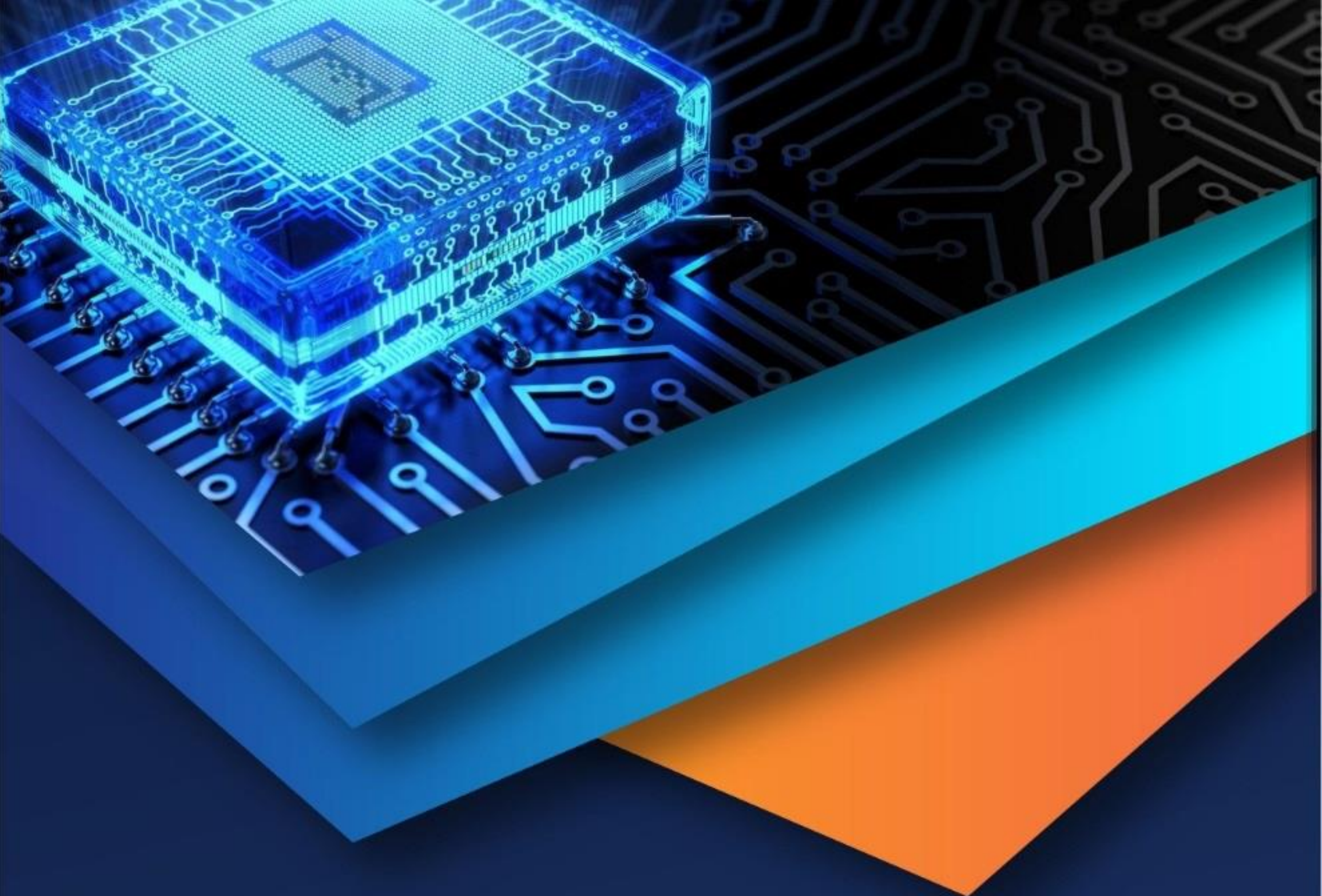

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