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Cellular composition of the Corpus luteum of Indian fruit bat, *Rousettus leschenaulti* (Desmarest) during early embryonic development

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ABSTRACT

The fine structure of luteal cell of the corpus luteum of Indian fruit bat, Rousettus leschenaulti was studied at three stages unilaminar blastocyst stage, Implanted bilaminar blastocyst stage and limb bud stage of early pregnancy. At unilaminar blastocyst stage luteal cells had small nuclei euchromatin. Mitochondria were small, round shaped with tubular cristae. Numerous less osmiophilic lipid droplets were observed in cytoplasmic field of the luteal cells. After implantation at implanted bilaminar blastocyst stage nuclear heterochromatin were reduced and nucleoli were larger and complex. Mitochondria were enlarged and often bizarre shaped with tubular cristae. Golgi complex and agranular endoplasmic reticulum were more conspicuous. Lipid droplets were less osmiophilic. At the stage of limb bud formation the luteal cells suggests different morphological picture, the nuclear size is reduced with clumps of heterochromatin. The agranular endoplasmic reticulum assumes the form of bundles of parallel tubules dispersed in several planes. Mitochondrial size was reduced then the previous stage and they posses vesicular cristae. These observations suggest that the steroidogenic activity of the luteal cells is highest during implantation and comparatively regresses during limb bud formation. It is suggested that the luteal cells is an important ovarian source of pregnancy hormones.

Introduction

The Indian fruit bat *Rousettus leschenaulti* has several peculiar characteristics including persistence of corpus luteum during pregnancy and several months after parturition. However, despite the prominent role that the corpus luteum plays in the embryonic development and the unusual persistence of corpus luteum there have been no ultrastructural studies of normal corpus luteum of *Rousettus leschenaulti.*

By contrast, many studies have appeared describing the fine structure of corpus luteum in other mammals including rabbit (Blanchette, 1966), pig (Cavazos et al ., 1969; Belt et al., 1970), cow (Priedkalns and Weber, 1968), and few bat species, *Miniopterus schreibersii fuliginosus* (Uchida et al., 1984), *Miniopterus sp* (Crichton et al., 1989; Bernard and Bojarki, 1994), *Macrotus californicus* (Crichton et al., 1990) and *Hipposideros lankadiva* (Seraphim, 2004),*Taphozous longimanus* (Nerkar and Gadegone 2007)during pregnancy. These studies have described the luteal cells as possessing abundant agranular endoplasmic reticulum mitochondria with tubular cristae osmiophilic lipid droplets, and a well developed Golgi apparatus. Furthermore changes in luteal ultrastructure have been observed to correlate strongly with luteal function.

The purpose of these investigations is to describe the fine structure of the corpus luteum of *Rousettus leschenaulti* at three stages of early pregnancy corresponding to progesterone level. This study should serve as a basis for further studies designed to elucidate the role of corpus luteum during early development of embryo.

Material and Methods

Small pieces of ovaries were cut and the tissue samples fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.3) for 12 hours at 4^0 C. After wash in buffer, the samples were post fixed in 1% OsO_4 for 2 hour at 4^0 C. The samples were dehydrated in an ascending grade of acetone, infiltrated and embedded in araldite CY 212 (TAAB, UK). Thick Sections (1 µm) were cut with an ultramicrotome, mounted on to glass slides, stained with aqueous toluidine blue and observed under a light microscope for gross observation of the area and quality of the tissue fixation. For electron microscope examination, thin sections of grey-silver colour interference (70-80 nm) were cut and mounted onto 300 mesh- copper grids. Sections were stained with alcoholic uranyl acetate and alkaline lead citrate, washed gently with distilled water and observed under a Morgagni 268D transmission electron microscope (Fei Company, The Netherlands) at an operating voltage 80 kV. Images were digitally acquired by using a CCD camera (Megaview III, Fei Company) attached to the microscope.

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Results

i) Unilaminar blastocyst stage

Ultrastructural studies on corpus luteum during early phase of pregnancy confirm that the luteal cells have the fine structural features of active steroidogenesis. At this stage, The granular endoplasmic reticulum (GER) consisting of short tubular cisternae and sometimes elongated profiles scattered in the cytoplasmic matrix. The relationship of cisternae of endoplasmic reticulum to the lipid droplets is seen near the nucleus.

The agranular endoplasmic reticulum (AER) is also present in large number in the lutein cells. Some luteal cells are studded with large polymorphic profiles of agranular endoplasmic reticulum. Mostly vesicular profiles of smooth endoplasmic reticulum are observed throughout the cytoplasm of luteal cells.

Mitochondria are abundant uniformly distributed throughout the cytoplasm. They are round in shape with tubular or vesicular cristae. The mitochondrial matrix is usually more electron dense than the cytoplasmic ground substance.

The luteal cells posses numerous randomly distributed large and small membrane bound lipid droplets. At this stage the luteal cells contain extremely less osmiophilic lipid droplets. The droplet profile is irregular and scalloped in contour. Sometimes large lipid droplets are packed with mitochondria. Some organelles like Golgi apparatus, free ribosome and lysosomes are unrecognizable. At this stage stromal cells are observed in the corpus luteum.

ii) Implanted bilaminar blastocyst stage

At this stage the luteal cells were large than the previous stage of pregnancy, with homogenous cytoplasmic matrix. Luteal cells presented somewhat variable appearance. Luteal nucleus is prominent with smooth nucleolemma. Some nucleus appears oval to round in shape contained prominent nucleolus and euchromatin is sparse in the nuclear matrix. Some luteal cells having irregular nucleus, without nucleolus and contained much heterochromatin adjoining the inner aspect of the nuclear envelope.

The granular endoplasmic reticulum (GER) consisting of short tubular cisternae is localized near the nuclear envelope and numerous stump like granular endoplasmic reticulum (rough endoplasmic reticulum) profiles scattered in the cytoplasmic field. The relationship of cisternae of endoplasmic reticulum to the mitochondria is seen in the cytoplasm.

The agranular endoplasmic reticulum (AER) is predominant organelle in luteal cell cytoplasm during this stage. In cytoplasmic areas which contained a

diverse collection of organelles, the agranular endoplasmic reticulum is found as a series of short anastomosing tubules or vesicles.

Mitochondria tended to concentrate in the central portion of the cell, but groups of mitochondria are also found throughout the cytoplasm. Mitochondria frequently clustered with lipid droplets and are often located outside the cisternae of the smooth endoplasmic reticulum and rough endoplasmic reticulum. They are round to elongate in shape. Mitochondrial cristae are tubular though in some cells cristae are lamellar.

Lipid droplets (Electron translucent) of different shapes and sizes are observed. Sometimes large lipid droplets are in close association with agranular endoplasmic reticulum and mitochondria. At this stage of development few stromal cells are observed in the corpus luteum tissue. These cells are located between the luteal cells and contained elongated nucleus. Golgi network and lysosomes are less prominent.

iii) Limb bud stage

At this stage of development luteal cells are smaller than the previous stages of early development. These cells are irregular in shape, with long expanding cytoplasmic processes extending between luteal cells of corpus luteum. The nucleus of these cells is variable in shape mostly round, oval, elongated or irregular in shape, sometimes with slight indentation. The nucleus is heterochromatic without nucleolus, nuclear membrane is clearly visible. Sometimes nucleus is eccentric in position.

The elongated profiles of granular endoplasmic reticulum arranged in parallel array, are observed near the nuclear membrane. Sometimes they are associated with mitochondria and agranular endoplasmic reticulum. Golgi apparatus is unrecognizable.

The cytoplasm of the cell is studded with polymorphic profiles of agranular endoplasmic reticulum, but the cytoplasmic field is reduced than the previous stages of pregnancy. The dense tubular form of smooth endoplasmic reticulum forming whorl near the nuclear envelope.

Mitochondria are spherical in shape, one or two elongated mitochondria suggest presence of some rod shaped mitochondria. The mitochondria have vesicular cristae with electron dense matrix. Few lipid droplets are observed in close association with smooth endoplasmic reticulum and mitochondria. Stromal cells are also visible and the nucleus of stromal cell is highly electron dense.

Ultrastructure of these three stages suggest that, before implantation cells contain polymorphic profiles of AER, mitochondria and lipid droplets after implantation cells are highly active, and contained numerous steroidogenic organelles (Agranular endoplasmic reticulum, mitochondria and lipid droplets)

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required for steroid synthesis. At limb bud stage organelles are reduced in number than the previous stage.

Discussion

The luteal cells of *Rousettus leschenaulti* during early pregnancy (Implanted blastocyst stage) possess large euchromatic nuclei. A close association of hypertrophied mitochondria with lipid droplets and anastamosing agranular endoplasmic reticulum in luteal cells is regarded as the characteristic features of steroidogenic cell are seen in the luteal cells of *Rousettus leschenaulti*. Luteal cells indicate active synthesis of steroidal hormone during this stage of pregnancy. Similar ultrastructural features of luteal cells are reported in other bat species, *Miniopterus schreibersii fuliginosus* (Uchida et al., 1984), *Miniopterus sp* (Crichton et al., 1989; Bernard and Bojarki, 1994), *Macrotus californicus* (Crichton et al., 1990) and *Hipposideros lankadiva* (Seraphim, 2004), during pregnancy.

In the present study the fine structure of luteal cells during early pregnancy shows short tubular cisternae of granular endoplasmic reticulum localized near the nuclear envelope and numerous stump like profiles scattered in the cytoplasmic matrix. Similar profiles of granular endoplasmic reticulum observed in, *Macrotus californicus*, (Crichton, et.al., 1990). In *Miniopterus schreibersi* (Crichton et al., 1989) polymorphic profiles of granular endoplasmic reticulum were observed. In *Taphozous longimanus* (Nerkar and Gadegone, 2007) few cisternae of granular endoplasmic reticulum with moderate abundance of ribosomes were observed.

Completely emptied lipid droplets in lutein cells were observed in few non-chiropterans, rabbit (Blanchette, 1966) humans (Adams and Hertig, 1969) and deer (Sinha, Seal and Doe, 1971). An interstitial cell of early pregnancy also shows electron lucent lipid droplets in rabbit. (Davies, and Broadus, 1968) supporting the present observation.

Conclusion

From present investigation it is evident that the corpus luteum synthesizes and secretes variety of proteins and peptide hormones which plays role in autocrine and paracrine functions, influencing luteal development remodelling and sterroidogenesis during early stages of pregnancy.

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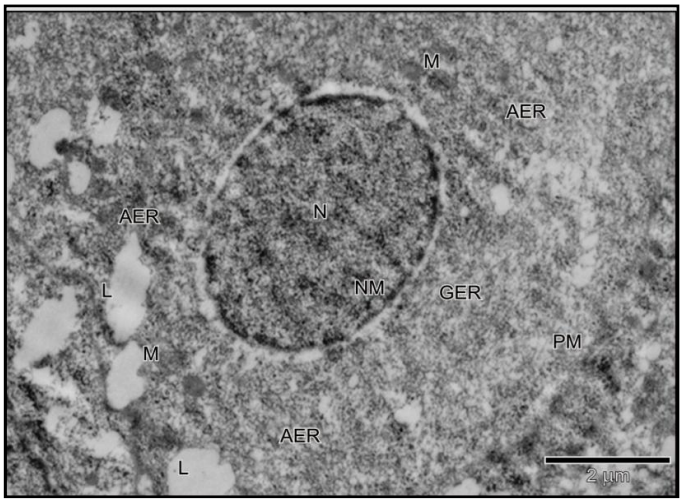
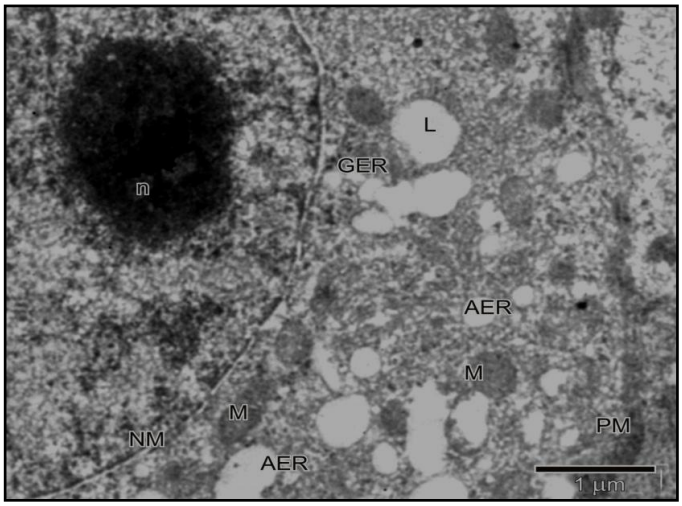
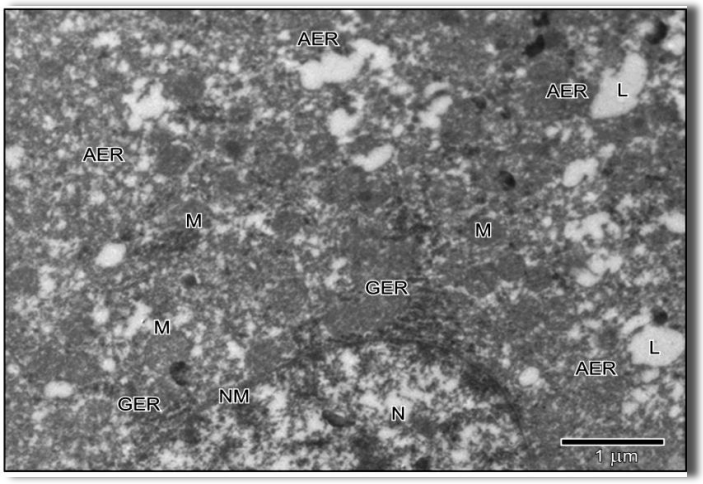
Observation Slides



I. Unilaminar blastocyst stage



II. Implanted bilaminar blastocyst stage



III. Limb bud stage (PM- Plasma membrane, AER- agranular Endoplasmic reticulum, GER- granular Endoplasmic reticulum, M- Mitochondria, L- Lipid Droplets, NM- Nuclear Membrane, N- Nucleus, n- Nucleolus)