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Ultrastructural studies on the Leydig cell of *Taphozous Kachhensis* (Dobson)

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ABSTRACT

Leydig cells constitute 4-6% of testicular volume and secrete the primary male sex hormone, testosterone. It is required to stimulate the male sexual differentiation, promotes male secondary sex characteristics, and maintains spermatogenesis. The smooth endoplasmic reticulum of interstitial cells reaches an unusual degree of development during sexually active period of *Taphozous kachhensis*. Approximation of SER and mitochondria with lipid droplets in cytoplasm of Leydig cell of *Taphozous kachhensis* indicates their role in the process of steroidogenesis. It has also been suggested that the extensive membranes of the agranular reticulum, in addition to providing sites for enzymes, may also act as a reservoir for the storage of cholesterol, since cholesterol is an important component of biological membranes. Leydig cells in *Taphozous kachhensis*, demonstrates endocytic/lysosomal pathway of steroidogensis. It shows coated pits, the uptake mechanism of LDL is evident at cell surface and early endosome (EE) and late endosome (LE) are identified. Ultrastructure of Leydig cell demonstrates, 'mylin figures' in close apposition with mitochondria and lipid droplets. These mylin figures are layered profiles of electron dense membrane and are significant in storing cholesterol in their compacted membranes.

Keywords - Leydig cell, mitochondria, lipid droplets, cholesterol

INTRODUCTION

The interstitial cells (Leydig cells) are clustered in the interstitium of testis, between seminiferous tubules. Leydig cells constitute 4-6% of testicular volume and secrete the primary male sex hormone, testosterone. It is required to stimulate the male sexual differentiation, promotes male secondary sex characteristics, and maintains spermatogenesis (Pudney, 1985). Through histolocalization techniques, it was found that, Leydig cells contains enzymes required for the process of steroidogenesis. (Pelletier et al., 2001; Haider and Trans., 2007; Miller and Auchus., 2010). Correlative histochemical and ultrastructural studies, indicated direct correlation exist between the amount of SER, lipid droplets and mitochondria in Leydig cells and

maximum steroid production in the testis (Payne and Hales, 2004).

Cellular components involved in the synthesis of androgens have been studied in the different seasonal mammals, guinea pigs (Christensen, 1965), the rat (Ichihara, 1993), the boar (Belt and Cavazos, 1967; Lunstra, et al., 1986), man (Kerr, 1991), and bat, *Myotis adversus* (Loh and Gemmell, 1980); *Taphozous georgianus* (Jolly and Blackshaw, 1989). Electron microscopic structure of Leydig cell were reported by Kent Cristensen (1965) in Guinea pigs; Connel and Christensen (1975) in canines; and Lunstra et al., (1986) in wild boar. Loh and Gemmell, (1980), reported seasonal changes in Leydig cell at ultrastructural level in seasonally breeding bat, *Myotis adversus*. In India, most extensive male reproductive study of emballunirid bat was undertaken by Singh, (1997) on *Taphozous longimanus.* There is no detailed information of ultrastructure of Leydig cell regarding its role in the reproduction of this bat. Thus, it would of interest to see if similar mechanism for the secretion of testosterone for the Leydig cell of the bat, *Taphozous kachhensis* during annual reproductive cycle.

MATERIAL AND METHODS

Adult male Indian flying fox, *Pteropus giganteus giganteus* were collected from Bramhapuri forest range, 20º21'52.45"N and 79 º53'37.33"E (Maharashtra, India). The testes were fixed by perfusion via testicular artery using 5% gluteraldehyde solution buffered with polyvinylpyrrolidone (PVP). Following 30 min. perfusion the tissue was cut into 1 mm blocks, immersed for an additional 30 min in fixative, post-fixed in 1% osmium tetroxide, rapidly dehydrated and embedded in epon. Thick and thin sections were cut on Leica Ultracut-R ultra microtome. Thick sections (1µ) were stained with toluidine blue and examined with light microscope to identify stages of the cycle of the seminiferous epithelium according to Leblond and Clermont (1952). Ultrathin sections were stained with uranyl acetate and lead citrate, and electro-micrographs of the sections were obtained by using Siemens JEOL-100S electron microscope at 100 KV accelerating voltage. All these electron microscopic methodologies were carried out in electron microscopy section of Jaslok Hospital, Mumbai.

RESULTS AND DISCUSSION

The sexually active period of annual reproductive cycle of *Taphozous kachhensis* was assessed by investigating fluctuations in morphological and hormonal parameters. Testis shows, reduced interstium which comprises Leydig cell alongwith fibrocyte and lymphocytes.(Fig.1) The nucleoplasm of Leydig cell shows, lightly stained uniformly distributed euchromatin dispersed at some places by darkly stained heterochromatin.(Fig.1) The most striking feature of Leydig cell during sexually active period is the hypertrophy of smooth endoplasmic reticulum.(Fig.2) In few sections, the agranular reticulum in the peripheral areas of the cytoplasm is arranged in a concentric whorls, containing 8-9 parallel running tubules. The concentric whorls are arranged around a common centre, encircling among them lipid droplet, mitochondria and few coated vesicles (Fig. 3). Spherical mitochondria with tubular lamelliform cristae are observed aggregate heavily in proximity to the site of energy production along with lipid droplets, smooth endoplasmic reticulum. (Fig.4)

Leydig cell of Taphozous kachhensis during sexually active period shows endosome/lysosomal system in the peripheral cytoplasm. (Fig.5) Early endosome then late endosome and coated vesicles were demonstrated by the hypertrophied Leydig cell. In the present study, Leydig cell cytoplasm of *Taphozous kachhensis* during the sexually active period shows, the lipofuscin granules generally contain three components : (1) a dense components in which dense core is surrounded by slightly lighter component, here interpreted as lipofuschin pigment (Fig.6) (2) a lipid droplet about 1 µ in diameter; and (Fig.6) (3) a cap like structure, which has a granular matrix and contains linear patterns in small patches (Fig. 6). The bounding membrane occurs on the outside of both the pigment and the cap and it seems likely that this membrane also extends over the lipid droplets to enclose the whole lipofuscin granules. In few sections an existence 'myelin figures' noticed. (Fig.6).

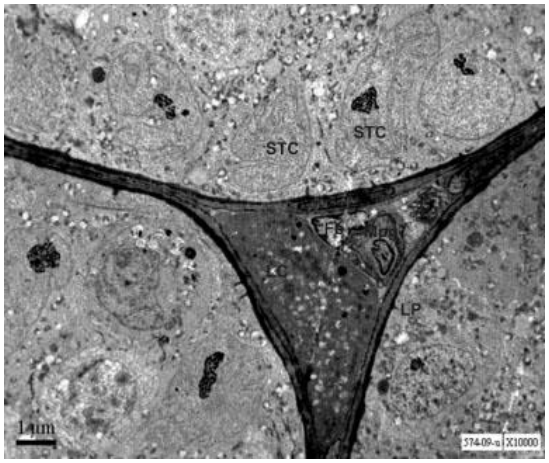

Fig. 1. Electron micrograph of the testis of *Taphozous kachhensis* during sexually active period, demonstrating triangular area of interstitium having fibrocytes (Fb), macrophages (Mpg) and Leydig cells (LC) bounded on all its side by the basement membrane of the seminfeorus tubules lumina propria (LP) (X10,000)

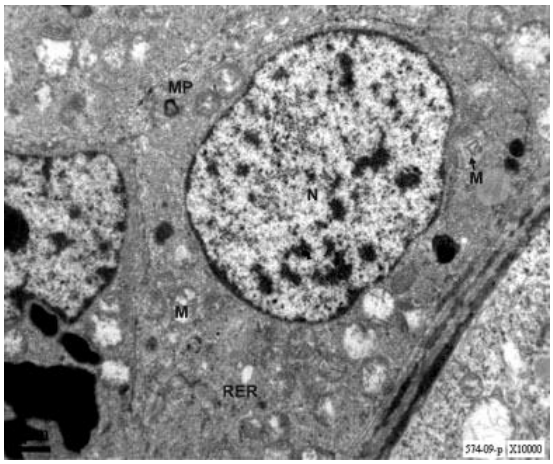

Fig. 2. Electron micrograph of the Leydig cell of *Taphozous kachhensis* during sexually active period, The nucleus (N) is displayed to one corner of the cell, slightly oval in shape, but may be irregularly contoured, lightly stained euchromatin dispersed randomly throughout the nucleoplasm. Anastomizing network of smooth endoplasmic reticulum (SER) prominently observed along with some microperoxisome like structure (MP), Profiles of spherical mitochondria (M) with collapsed tubular cristae also seen. Note the presence of endosome iln the ectoplasm of the Leydig cell. (10,000)

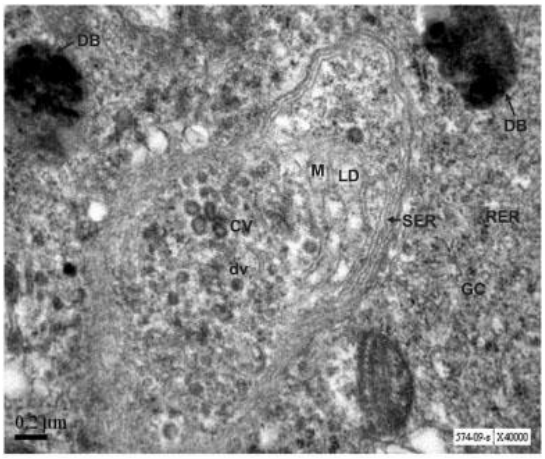

Fig. 3. Electron micrograph of the Leydig cell of *Taphozous kachhensis* during sexually active period, illustrating the agranular reticulum (SER) in the peripheral areas of the cytoplasm is arranged in a concentric whorls, containing 8-9 parallel running tubules. The concentric whorls are arranged around a common center, encircling among them lipid droplet (LD), mitochondria (M), few coated vesicles (cv), dense vesicles (dv) and microtubules (mt) . Mixed profiles of RER and SER in the form of anastomising network observed. Also seen electron dense lipofuschin pigment granules in dense bodies (DB) (X40,000)

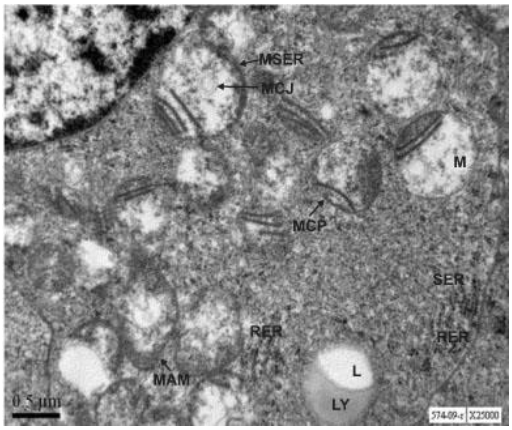

Fig. 4. Electron micrograph of the Leydig cell of *Taphozous kachhensis* during sexually active period, illustrating interconnected network of smooth endoplasmic reticulum (SER) thoughout the matrix substance of the cytoplasm. Large number of spherical mitochondria (M) with lameliform tubular cristae and elecro-lucent osmophilic granules disperse in the matrix, Mixed profiles of interconnected network of smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER) seen dispersed thoughout the cytoplasm. Also observed in the section mitochondrial associated membrane (MAM), contact point region of outer and inner mitochondrial membrane (MCP), association of mitochondria and SER (MSER), lamellar association between tubular cristae (LA). Also seen Lipid droplet (LD) fused with secondary Lysosome (LY), forming cap- like structure (X 25,000)

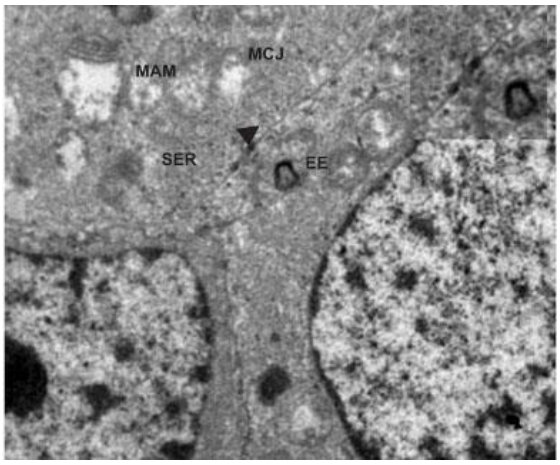

Fig. 5. Electron micrograph of the Leydig cell of *Taphozous kachhensis* during sexually active period, demonstrating junctional complexes (JC) between the plasma membrane of adjacent Leydig cells like gap junctions, rudimentary desmosomes and surface indentations. Darkly stained heterochromatin found interspersed at places throughout the euchromatin nucleoplasm, some of which found attached to the inner profile of nuclear membrane. s(X 15,000)

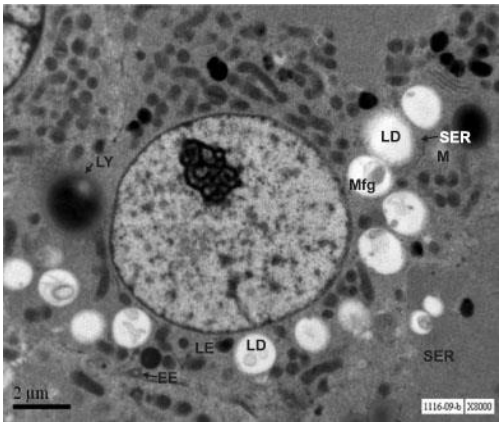

Fig. 6. Electron micrograph of the Leydig cell of *Taphozous kachhensis* during sexually active period, demonstrating ectoplasm having anastomising network of smooth endoplasmic reticulum (SER) forming the ground substance of the cytoplasm. Spherical mitochondria (M) seen in close association with the SER (MSER) and it also shows narrow circular junctions of cristae to open into inner matrix called as crista junctions (MCJ) and mitochondrial associated membrane (MAM). Inset shows junctional complex between the cell membrane of two adjacent Leydig cells. Note the presence of coated pits (cp) and coated vesicle (cv). (X30,000)

DISCUSSION

In the bat, *Taphozous kachhensis* increased concentration of plasma testosterone during sexually active period correlated with changes in the ultrastructure of Leydig cell. The smooth endoplasmic reticulum of interstitial cells reaches an unusual degree of development during sexually active period of *Taphozous kachhensis*. These findings during present investigations similar to that illustrated in previously on different mammalian species, guinea pig (Christensen, 1965); Squirrel monkey (Belt and Cavazos,1967); bat, *Rhinolopus capensis* (Bernard., 1986); ground squirrel, (Pudney, 1986). Histochemical studies, demonstrated the function of these membranes of the agranular reticulum as a site of many steroidogenic enzymes (Pudney, 1985; Zircin, 2000; Pelletier et al., 2001; Payne and Hales, 2004; Haider et al., 2007; Miller and Auchus, 2011).

Approximation of SER and mitochondria with lipid droplets in cytoplasm of Leydig cell of *Taphozous kachhensis* indicates their role in the process of steroidogenesis. It has also been suggested that the extensive membranes of the agranular reticulum, in addition to providing sites for enzymes, may also act as a reservoir for the storage of cholesterol, since cholesterol is an important component of biological membranes (Stocco,1996; Zircin, 2000; Pelletier et al., 2001). Steroidogenic acute regulatory protein (StAR) and peripheral benzodiazepine receptors (PBR) transfer cholesterol from the outer membrane to the inner mitochondrial membrane (Pelletier et al.,2001; Stocco, 2007; Miller and Auchus, 2011). The enzyme P450 side chain cleavage, residing on the matrix side of the mitochondrial inner membrane, converts cholesterol into prognenolone, which is ultimately transferred to the smooth endoplasmic reticulum, where testosterone is synthesized by a series of steroidogenic enzymes. (Payne and Hales, 2004; Stocco et al., 2007; Miller and Auchus, 2011).

The mixed profile of RER and SER, in approximation with mitochondria and Golgi complex reflects their involvement in synthesis of proteins including enzymes, transport and binding proteins, carrier proteins for steroids and various regulatory peptides including growth factors etc. (Hall, 1994; Payne and Hales., 2004; Miller and Auchus, 2011).

Leydig cell in *Taphozous kachhensis* shows mitochondrial associated membrane (MAM) and Lamellar associations (LA). The lamellar association (LA) of the cristae appears unique in steroid producing cells. (Prince and Buttle, 2004). These lamellar association (LA) between the tubular cristae of mitochondria do not involved in the ATP synthesis. (Prince and Buttle, 2004). The LA suggested as a temporary morphology representing a specific step during steroidogenesis. (Prince and Buttle, 2004) The possible significance of LA is found with one lamella in close apposition with inner boundary membrane (IBM) and formation of cristae junction (CJ) (Prince and Buttle, 2004). Cristae junction is implicated in the translocation of cholesterol as they are the contact point between inner boundary membrane and outer mitochondrial membrane (OMM). (Pfanner, 1990)

Cholesterol can be synthesized de novo, mobilized from stored cholesterol esters (lipid droplets) or obtained through the uptake of low density cholesterol (LDL) (Prince, 2007). In the present investigation, Leydig cells in *Taphozous kachhensis*, demonstrates endocytic/lysosomal pathway of steroidogensis. It shows coated pits, the uptake mechanism of LDL is evident at cell surface and early endosome (EE) and late endosome (LE) are identified. The pathway of endocytosed material through endosome to the lysosomal and Golgi compartment has well demonstrated by the Hermo et al., 1985. Ultrastructure of Leydig cell demonstrates, 'mylin figures' in close apposition with mitochondria and lipid droplets. These mylin figures are layered profiles of electron dense membrane and are significant in storing cholesterol in their compacted membranes. (Prince, 2007).

CONCLUSION

In conclusion, ultrastructure of Leydig cell of *Taphozous kachhensis* shows, hypertrophy of cellular organelles can be correlated with its role in steroidogenesis. Extensive development of smooth endoplasmic reticulum, mitochondria in approximation with lipid droplets indicates its involvement in trafficking of cholesterol which is precursor molecule for steroid synthesis. Endosomal pathway for mobilization of steroid precursors also seen in the electron microscopic structure of Leydig cell. Histolocalization of steroidogenic enzymes in the Leydig cell of this bat, will be the topic of future investigation.

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