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## Electron Microscopic Studies of the Adrenal Gland during Seasonal Cycle of Fruit Eating Bat Pteropus *Giganteus giganteus*

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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#### ABSTRACT

The fine structure of the adrenal gland is testified in many mammals; whereas, very diminutive information is available on the fine structure of the bat's adrenal gland. The present work is constrained to the ultrastructural studies of the adrenal gland of *Pteropus giganteus giganteus*. The adrenal gland of the male bat has two distinct perceptible zonations viz., cortex and medulla. The cortex is divided into three illustrious zones namely, zona glomerulosa, zona fasciculata and zona reticularis. The differences in the zonation under the electron microscope becomes more acute when the studied bat is sexually active. Histoarchitectural study of the adrenal gland designate prominent structures and striking differences in the adrenocortical zone during the sexually active period than the sexually quiescent period. The zona fasciculata is well developed during the sexually active period than that of the sexually quiescent period. Likewise, zona fasciculata is well

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developed as compared to zona glomerulosa and zona reticularis of the adrenal cortex in both active and quiescent stages. Light microscopic studies on adrenal gland are confirmed by the ultrastructural studies.

Keywords: Pteropus; Zona glomerulosa; Zona fasciculata; Zona reticularis; ultra structure.

#### 1. INTRODUCTION

The data of the measurement of the adrenal functions in the chiroptera is reported only from few species. These results indicate that there are both seasonal and stress-related changes in the adrenocortical activity (Chavhan et al., 2011; Gustafson and Belt, 1981; Widmaier and Kunz, 1993; Widmaier et al., 1994, Shende V A, etal 2014). The seasonal changes are also reported in the adrenal morphology of the chiroptera. The role of the adrenal gland in the metabolism and homeostasis of the mammals has been widely investigated (Shier D, etal 2007). The mammalian adrenal gland is unique among vertebrates as the steroidogenic and chromaffin cells are clearly separated as cortex and medulla respectively. Contemporarily, the ultrastructural findings have insighted in to the morphological features of the normal adrenal gland of a number of mammals viz., rat man and guinea pig and Indian grey mongoose, Indian Sheath- Tailed Bat (Johannsson, 1968; McNutt, et al., 1970; Nishikawa et al., 1963; Sheridan, et al., 1964; Shetty, et al., 1984).\_Nerkar, M M Gadegone (2012).

The structure of the adrenal gland of a megachiropteran bats namely, Cynopterus sphinx and Taphozus longimanus has been reported earlier (Brenner, 1966). The distinct zonation of the cortex and the demarcation of the medulla is reported in Miniopterus schreibersii (Karim, et al., 1979), Megaderma lyra lyra (Brown et al., 1970) and Rousettus leschenaulti and Pteropus giganteus giganteus (Planel, et al., 1961). In C. Sphinx, the adrenal cortex has three distinct zones. The medulla of the adrenal gland in both species consists of cells arranged in a small groups or short cords surrounded by the blood capillaries.

In rhesus monkey and male *Pteropus* bat, the mitochondria have lamelliform cristae in the zona glomerulosa and tubular cristae in the both, zona fasciculata and zona reticularis. Agranular reticulum of the tubular form is rare in the zona glomerulosa but in great abundance in the fasciculata and reticularis. The cells of zona fasciculata contained large and highly ordered

"stacks" of the granular endoplasmic reticulum, which showed multiple connections with the random network of tubular agranular reticulum. The lipid droplets in the inner cortical regions were enveloped by the tubules of the agranular endoplasmic reticulum (Bhima Rao et al., 1975). The medullary cells of *Pteropus giganteus giganteus* during the sexually quiescencet, breeding phases are loaded with epinephrine and norepinephrine secretory granules. The present study specifically encircles on the cortex of the *Pteropus giganteus giganteus* by means ultrastructural approach.

#### 2. MATERIALS AND METHODS

#### 2.1 Transmission Electron Microscopy

**Fixation:** The adrenal glands were selected for the electron microscopic studies during the sexually breeding and sexually quiescent period. They were sliced into 2 mm pieces and immersed in fresh, ice-cold 3% glutaraldehyde solution. The tissues were then washed in the cold 0.1 M sodium cacodylate buffer over half an hour. After fixation with OsO4, an osmification process with 1% OsO4 in sodium cacodylate buffer was carried out.

**Dehydration:** The dehydration of the tissue was carried out by passing the fixed tissue through a series of graded ethyl alcohol. Thus prior to embedding, the tissues were passed through two changes of a transitional solvent preceding to propylene oxide over a period of half hour.

**Infiltration and Embedding:** Embedding of the tissue blocks was done in the Propylene oxide: Araldite 'A' solution 1:1 for one hour at room temperature BEEM capsule with fresh araldite; the capsules were kept in an oven maintained at  $60^{\circ}$ C for 24 to 48 hours to complete polymerization. The blocks were trimmed with a safety razor blade under the stereomicroscope. Semi-thin sections of 1 to 2µ in thickness were cut on the LKB Ultra tome V with glass knives prepared on the LKB 7800 B knife markers. These sections were dried on a hot plate ( $60^{\circ}$ C) and stained with 1% toluene blue and observed on the light microscope. The selected areas for the ultrathin sections were marked out.

The blocks were further trimmed to the ultrathin sections having 600-900 A<sup>o</sup> thickness. The pale gold colour was cut and sections were collected on 300 mesh copper grids. To enhance the contrast a double staining technique was employed. The grids were subjected to 10% alcoholic uranyl acetate for half an hour followed by the lead citrate. All grids were observed on a JEOL -100S Electron microscope at 80KV accelerating voltage. The microphotographs of the desired samples were taken at different anticipated planes and different magnifications.

#### 3. RESULTS

#### 3.1 Electron Microscopic Structure of Adrenal Gland

#### 3.1.1 Adrenal gland during sexually quiescent period

The adrenal gland shows an outer cortex is clearly divided into three zones namely, zona glomerulosa, zona fasciculata and zona reticularis and medulla.

Zona glomerulosa - It is the outermost zone of the cortex with spherical nucleus having welldeveloped nuclear membrane at centric or eccentric position. The nucleus shows clumps of chromatin material along the rim of the nuclear membrane (Fig. 9). The mitochondria show various shapes like spherical and elongated to oval, having lamelliform and vesicular cristae. The cytoplasm shows a granular endoplasmic reticulum in the form of small vesicles along with many freely scattered ribosomes. The Golgi complex consists of many dilated saccules and smooth endoplasmic reticulum (SER). Numerous small and medium sized lipid droplets were observed in the cytoplasm. The multivesicular bodies containing formative dense bodies and lysosomal bodies were observed in the cytoplasm.

**Zona fasciculata** - It occupies most of the part of the cortex. The nucleus is at eccentric in position, along with granular nucleoplasm and patches of heterochromatin. Large oval to spherical mitochondria and cristae was lamellar as well as vesicular. The dilated golgi saccules were arranged in the circle. In centre of the lamellae electron dense granules were enclosed and in vesicles or vacuoles too contains dense

granules. Few cisternae of rough endoplasmic reticulum (RER) were observed. Many ribosomes were seen freely in cytoplasm and SER are also observed in the same (Fig. 11 and 12). The presence of numerous lipid droplets was also observed. Dense vacuolated oval and round shaped bodies were finely granular.

**Zona reticularis** - The cells are polygonal in shape having homogenous cytoplasm along with spherical and oval to elongated nucleus was placed eccentrically in the cell (Fig. 11). The lipid droplets were less in number and smaller than those lipid droplets present in the cells of the fasciculata. The Golgi complex is inconspicuous. The mitochondria were oval to elongate in shape and were few in number. The ribosomes were free in the cytoplasm along with RER. (Fig. 10).

**Medulla -** It is the inner most zone of the adrenal gland composed of cords of chromaffin cells. These cells were irregular in shape and eccentrically placed in the nucleus with peripheral chromatin clumps. The cytoplasm of these cells contains unevenly distributed chromatin vesicles. The Golgi complex was inconspicuous. The RER consisted of vesicular cisternae and the mitochondria was large vacuolated with lamellar cristae. Few lysosomal and multivesicular bodies were observed in cytoplasm (Fig. 13).

### 3.1.2 Adrenal gland during the sexually active period

Zona glomerulosa - It is the long-looped cords of cells covered with capsule. The nucleus is oval, elliptical to irregular in shape and eccentric in position. The mitochondria are numerous, oval to spherical in shape and dispersed in the cytoplasm along with transverse and vesicular cristae. The RER was well developed with ribosomes. The Golai complex was well developed and the lamellae was parallelly arranged. In the cytoplasm, few large and medium sized electron lucent lipid droplets were observed. Numerous lysosomes and complex dense bodies were also seen in the cytoplasmic matrix. The SER in the form of vesicles was distributed throughout the cytoplasm (Fig. 14).

**Zona fasciculata** - It occupies most of the part of the cortex. These cells were larger than those cells of the zona fasciculata observed during the sexually quiescent period. The nucleus was oval to irregular and placed eccentrically in the cell. The chromatin material was present along the

(Fia. 15). inner nuclear membrane The cytoplasm of the cell was filled with moderately dense lipid droplets and were higher in number than those of the zona fasciculata of the sexually quiescent period. Numerous ovals and elongated shaped mitochondria with vesicular cristae were present in the cytoplasm. The Golgi complex was prominent and its lamellae arranged in the semicircular and circular form enclosing the electron dense vesicles. The lipid droplets were observed in the close association with the Golgi complex and mitochondria. The SER and the lipid droplets play a very important role in steroidogenic function. The lysosomal bodies were present in the cytoplasm. This type of association as not observed in the cells of the in sexually quiescent period fasciculata (Fig. 15,16).

**Zona reticularis** - The reticularis cells were smaller than those of the cells of the zona fasciculata. There were numerous ovals to round

mitochondria with sparse matrix. The cells show larger in number of the lipid droplets than that of observed in the reticularis of the sexually quiescent period. The lipid droplets were distributed throughout the cytoplasm. The profiles of RER were observed in the cytoplasm. Many lysosomal bodies and well-developed Golgi complex were seen in cytoplasm (Figs. 17,18).

**Medulla -** The chromaffin cells were different in terms of shape, size and density, with irregular outline. The cytoplasm of these cells contained chromaffin vesicles. The network of the RER was observed, which was in the form of few cisternae and tubules. The oval to spherical mitochondria with few transverse cristae or peripheral cristae was dispersed in the cytoplasm. The free ribosomes were numerous and gave a granular appearance to the cytoplasm. The SER was inconspicuous. Lipid droplets and lysosomes were few, but present in the cytoplasm (Fig.19).

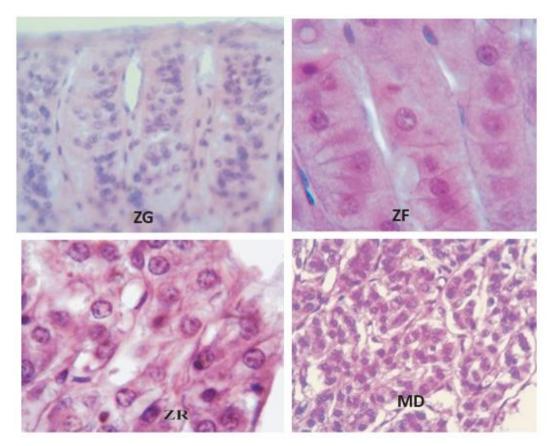


Fig. 1 to 4. Transverse section of adrenal gland during sexually quiescent period of reproductive cycle shows the presence of outermost capsule (CP) followed by large acini like group of cells of zona glomerulosa (ZG), long cords of zona fasciculata (ZF), network of cells of zona reticularis (ZR showing inner small cords of cells in medullary zone (MD). X 1000

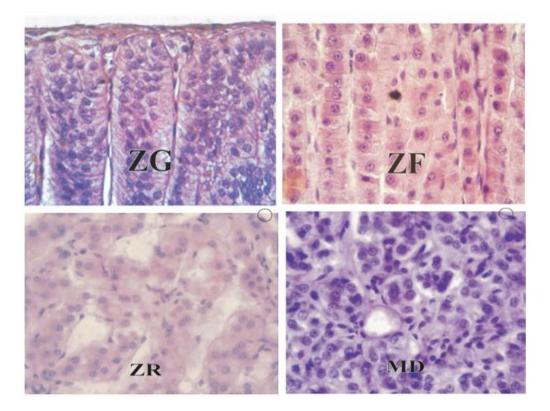


Fig. 5 to 8. Transverse section of adrenal gland during sexually active period of reproductive cycle shows the presence of outermost capsule (CP) followed by long acini like group of cells of zona glomerulosa (ZG), hypertrophied cell cords of zona fasciculata (ZF) with vacuolations, cells of zona reticularis (ZR), enlarged medullary zone (MD). X1000 In light microscopy double by staining method two different stains used as Haematoxylin and eosin

#### 4. DISCUSSION

At the ultrastructural level the adrenal gland of the sexually active and sexually quiescent *Pteropus* shows striking differences in the adrenocortical zone\_(Belloni etal 1987). The zona fasciculata has been found to be well-developed during the sexually active period than that of the sexually quiescent period. Furthermore, zona fasciculata was also well-developed as compared to zona glomerulosa and zona reticularis of the adrenal cortex in both active and quiescent stages.

glomerulosa: During Zona the sexually quiescent period, the mitochondria has shown various shapes viz., spherical and elongated to oval, having lamelliform and vesicular cristae. The observed cells possess agranular endoplasmic reticulum in the form of small distributed vesicles and many ribosomes were seen freely scattered in the cytoplasm. The Golgi complex consists of many dilated saccules. The RER was in the form of short stump dotted with ribosomes. Large numbers of small and medium sized lipid droplets were observed in the cytoplasm. The lysosomal bodies were also seen in the cytoplasm. Similar observations have also been reported in *H. lankadiva* and *Taphozous kachhensis* (Brown, et al., 1970; Chavhan, et al., 2011).

The ultrastructural characteristics of the cell of the zona glomerulosa during sexually active phase shown numerous mitochondria with oval to spherical in shape dispersed in the cytoplasm having transverse and vesicular cristae. The cisternae of the RER were dotted with ribosomes. The Golgi complex has been found to well-developed with parallelly arranged be lamellae. In the cytoplasm few large and medium sized electron lucent lipid droplets were observed. Numerous lysosomes and complex dense bodies were also seen in the cytoplasmic matrix. The SER was in the form of vesicles distributed throughout the cytoplasm. A SER in the form of small vesicles was also seen nearby mitochondria and lipid droplets. In the rat, the alomerulosa cells were denser and contains free ribosomes and few elements of the vacuolar system (Dhamani, 2004). The network of the endoplasmic reticulum was predominantly smooth-surfaced with cluster of ribosomes were observed near membrane. The cisternae of the SER were present along with the lipid droplets and has been confirmed by other workers too (Sabatini, et al., 1961; Wheatley, 1968) In the giganteus, Pteropus giganteus the zona glomerulosa shown large and medium sized lipid droplets in larger in numbers during the sexually active period than that of sexually quiescent period.

**Zona fasciculata:** The observed cells were cuboidal to polygonal in shape and were arranged in the long cords. The nucleus was oval to irregular in shape and eccentrically placed. The cytoplasm was filled with numerous mitochondria with spherical to oval in shape

having vesicular cristae. In some mitochondria, the cristae were unevenly distributed, but grouped at varying intervals into the clusters with less electron dense matrix. Similar observations were also reported in many mammals where mitochondria were of varying size and shapes in zona fasciculata (Friend et al., 1970; Dhamani, 2004; Coupland, 1965).

In the *Pteropus giganteu giganteus*, the zona fasciculata cells has shown a close association among SER, lipid droplets and mitochondria during sexually quiescent and sexually active phase, which has been also reported in similar works (Dhamani, 2004; Kadioglu, et al., 1975; Sabatini et al., 1961). The Golgi complex and RER were inconspicuous. The Ribosomes were fewer in number and confirmed by other too (Nishikawa et al., 1963).

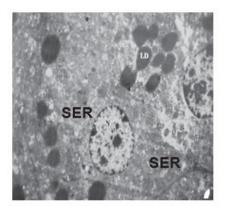


Figure- 9 ZG

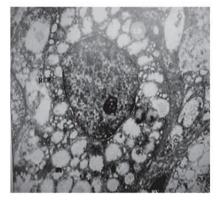


Figure-10 ZR



Figure- 11 ZF

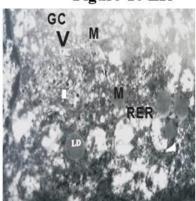
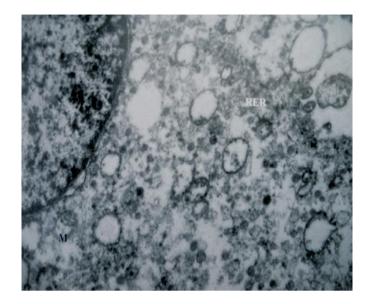


Figure- 12 ZF

Fig. 9,10,11,12. Electron microscopic structure of zona glomerulosa (ZG), zona fasciculata (ZF) zona reticularis (ZR) cells of adrenal gland during sexually quiescent period showing prominent nucleus (N), nucleolus (NU), several lipid droplets (LD), Rough endoplasmic reticulum (RER), Lysosome (LY), Mitochondria (M) Golgi complex (GC) and lipid droplets (LD), vacuole (V), vesicles (VE) respectively



## Fig. 13. Magnified view of electron microscopic structure of adrenal gland medulla during sexually quiescent period showing medulla (MD) with nucleus (N), rough endoplasmic reticulum (RER), mitochondria (M) with transverse cristae and secretory granules (SG)

In the sexually quiescent and sexually active period, the observed cells of the zona fasciculata showed large sized lipid droplets, but the number during the sexually active period is more than that in sexually quiescent period. Similar observations were reported in other mammals (Sabatini et al., 1961; Lever, 1956).

In the sexually active period, the cytology of the zona fasciculata has shown striking features such as elaborate development of the SER, mitochondria with vesicular cristae and large droplets. number of lipid All these observations suggest the increased rate of synthesis activity in the cell of the zona sexuallv fasciculata durina the active phase. The close association of the SER with mitochondria and lipid droplets (Tokar, etal 2004) indicates that these cells must have been engaged in steroid biosynthesis and interconversion.

**Zona Reticularis -** It is present below to zona fasciculata and adjacent to the medulla. The lipid droplets were less in number and smaller in size. The Golgi complex was inconspicuous. The

mitochondria were oval to elongate in shape and few in number. The ribosomes were free in cytoplasm. The RER was observed in sexually quiescent phase. The number of lipid droplets were observed to be less in the reticularis during the sexually quiescent phase. The lipid droplets were distributed throughout the cytoplasm of cells. The profiles of RER were observed in the cytoplasm. Many lysosomal bodies were seen in the cytoplasm. The Golgi complex was welldeveloped in sexually active phase.

During the sexually active phase the lipid droplets were larger than those observed during sexually quiescent phase. It was assumed that high rate of the protein synthesis was occurring stacks. within these There was no cytological evidence of the protein being made or export in the adrenal cells. The proteins manufactured in the stacks may be enzymes needed in the adrenal cortical cell's metabolism. Since the stacks were in the direct continuation with the tubules of the agranular endoplasmic reticulum, where steroidogenesis occur, the enzyme made may be involved in steroidogenesis (Bhima Rao et al., 1975; Kadioglu et al., 1971).

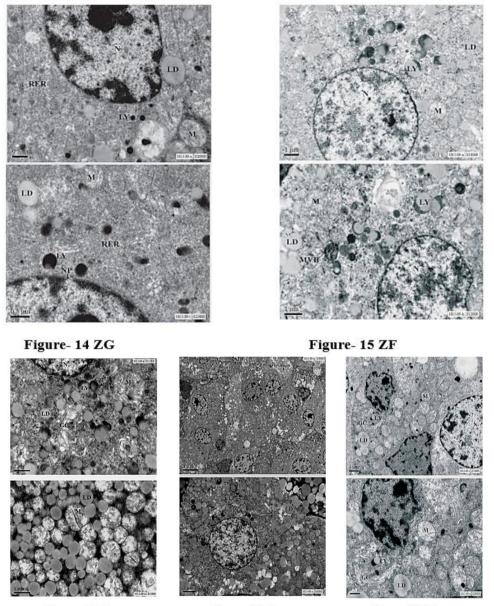


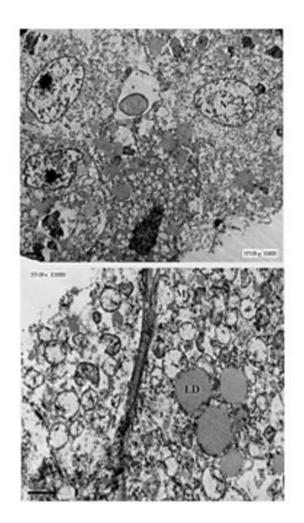
Figure- 16 ZF

Figure- 17 ZR

Figure- 18 ZR

#### Fig. 14 to 18. Electron microscopic structure of zona glomerulosa (ZG), zona fasciculata (ZF) zona reticularis (ZR)cells of adrenal gland during sexually active period showing well developed plasma membrane (PM), nucleus (N), lipid droplets (LD), rough endoplasmic reticulum (RER), mitochondria (M) are observed well developed lysosomes (LY), lipid droplets (LD) are seen smooth endoplasmic reticulum (SER), junctional complex (JC)

**Medulla -** It occupies the centre of adrenal gland cells having polygonal shape with oval to irregular nucleus. These observations can be confirmed with the observations reported in other mammals' polygonal cells having large eccentric nuclei with two to five nucleoli having diffuse granular nucleoplasm and granules in cytoplasm (Bloodworth et al., 1968; Long, et al., 1967; Yýlmaz et al., 2005). The sinusoids were located in between the chromaffin strands, which consist of chromaffin cells (Coupland, 1965a; Yýlmaz et al., 2005). The Golgi complex consists of many dilated saccules. The RER consists of vesicular cisternae. The mitochondria were large vacuolated with lamellar cristae. In some, peripheral cristae were observed. The few lysosomal and multivesicular bodies were observed in the cytoplasm during inactive phase. The Golgi complex was inconspicuous, but many Golgi vesicles were seen dispersed in the cytoplasm of medullary cells of *Pteropus giganteus giganteus*.



#### Figure- 19 MD

# Fig. 19. Electron micrograph of medullary (MD) cells of adrenal gland during sexually active period. Note the nucleus (N), mitochondria (M), electron dense secretory granules (SG), lipid droplets (LD)

#### 5. CONCLUSION

The adrenal gland during the sexually active phase of *Pteropus giganteus giganteus* shows significant differences of histoarchitecture when compared to the sexually quiescent phase. The ultrastructural characteristics of the adrenal cortex cells suggest their participation in the steroid biosynthesis and interconversion along with synthesis of proteins. It supports the wellestablished fact that the primary function of the adrenal gland is to protect the organism against acute and chronic stress. The ultrastructural studies of the adrenal gland implies that there are both seasonal and stress related changes in the adrenocortical and medullary cells of *Pteropus giganteus giganteus*. The well-developed zona glomerulosa and fasciculata and enlarged medulla confirms an active participation of the adrenal gland during reproductive processes of the *Pteropus giganteus giganteus*.

The medullary cells of this bat during the sexually quiescent and breeding phases were loaded with epinephrine and norepinephrine secretory granules. The above reports make this bat an interesting model for further studies to find physiological role of endocrine gland on reproductive timing.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### **COMPETING INTERESTS**

Author has declared that no competing interests exist.

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