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SEASONAL CHANGES IN THE ADRENAL GLAND OF THE INDIAN MALE FRUIT BAT *PTEROPUS GIGANTEUS GIGANTEUS* (BRUNNICH) DURING THE REPRODUCTIVE CYCLE

[1] J. N. PAPADKAR AND [2] A.A.DHAMANI

[1]DEPARTMENT OF ZOOLOGY, M. G. COLLEGE, ARMORI (M.S.)

[2] DEPARTMENT OF ZOOLOGY, N. H. COLLEGE, BRAMHAPURI (M.S.)

jpapadkar@gmail.com & dhamaniamir@gmail.com

ABSTRACT:

The present study shows the cyclical alternation in the activity of adrenal gland of the male bat *Pteropus giganteus giganteus* during different phases of reproductive cycle. The adrenal gland of *Ptreropus* is made up of the steroidogenic and chromaffin cells as in cortex and medulla respectively. The Indian fruit *bat P. giganteus giganteus* species has a sharply defined breeding season. This species experiences a single reproductice cycle. Adrenal glands of sexually active bats are significantly larger than that of other phases of reproductice cycle. The cortex is outermost zone completely encircles the central medulla . The medulla consists of polymorphic epitheloid cells arranged in small groups or short cords surrounded by blood capillaries. The zona glomerulosa consists of columns of cells with darkly stained nuclei each with a nucleolus. The Zona glomerulosa imperceptibly merges with zona fasciculata, which occurs in the form of cords of polyhedral cells which are large and vacuolated & are arranged radially. Each cord is only one or two-cell thick with sinusoidal capillaries separating them. The zona reticularis consists of a network of cellular cords. The nuclei of some cells are pycnotic, and contain a deposite of pigment. The cells are hypertrophied, cytoplasm is eosinophilic with darkly stained nucleus and more vacuolated.

KEY WORD: *Adrenal gland, Bat.*

INTRODUCTION:

Adrenal gland is a key organ in bat as it plays an important role in metabolism and homeostais of animals. In mammals, stress and hyper and hypo functions of this gland are known to suppress reproduction [14]. Although the data of measurement of adrenal functions in Chiroptera is reported in a few species, these results indicate that there are both seasonal and stress related changes in adrenocortical activity [11,13].

Mammalian adrenal gland is unique among the vertebrate in that steroidogenic and chromaffin cells are clearly separated as cortex and medulla respectively. The light microscopic observations of adrenal gland are known in few species of bats, viz., *Hipposideros lankadiva*[17], *Vesperugo pipistrellus*[10], *Rousettus leschenauti*[8] *Cynopterus sphix* and *Taphozous longimanus* [9], *Miniopterus schreiberscii*[1], *Taphozous Kachensis* [19].

The objective of present study is to analyse the histoarchitechtural changes in the adrenal gland of the Indian male fruit bat *Pteropus giganteus giganteus,* during different phases of the reproductive cycle. It has been undertaken because of its unusual reproductive behaviour, and also to find out the probable role of adrenal gland in the control of reproduction if any in this seasonally breeding bat.

MATERIALS AND METHODS:

Indian male fruit bat, *Pteropus giganteus giganteus* (Brunnich) found on trees hanging upside down near water sources. This bat was selected for the present study because of its unique reproductive habits. The specimens were collected from Padmapur 40 Kms. from Bramhapuri, District- Chandrapur in Maharashtra (India), throughout the year representing different cycle during (December 2006 to November 2009). They were brought to laboratory alive with minimum stress. The males are easily identified by their external genitelia and gloden-brown fur around neck. Mature and large sized adult males were caged for the experimant weight recorded with sensitive balance before they sacrificed. Bats are sacrificed by decapitation using anesthesia.

For histological examination adrenal gland from sexually inactive and sexually active bats excised out from the body cavity. The weight of adrenal was recorded separately by using highly sensitive monopan electric balance. The tissue fixed in alcoholic Bouin's fixative for 24 hrs, followed by preservation in 70% ethyl alcohol. The tissues were dehydrated through the graded series of ethanol, cleared in xylol and embedded in paraffin wax. The tissues were cut at 5 to 6 µ with the help of Rotary microtome. The sections were stained with haematoxylin and eosin for routine histological examination. The microphotographs were taken with the help of a Lobamade camera attached to the microscope and enlarged to the required size.

For morphometric analysis, sections were selected and measurements were taken with the help of an ocular micrometer. The diameter of the whole adrenal gland, diameter of medulla and width of the cortex, zona glomerulosa, zona fasciculata and zona reticularis during different phases of reproductive cycle were measured. The available data was analyzed by statistical method.

RESUTLS:

The paired adrenal gland of *Pteropus giganteus giganteus* is situated on each side retroperitonially occupying the antero-mesial position abutting against the ipsilateral kidney to which it is firmly attached

by renal fascial and is enclosed by thick capsule. The adrenal gland can be distinguished as an outer cortex and inner medulla. The cortex completely encircles the medulla which is relatively less extensive.

A. ADRENAL GLAND DURING SEXUALLY INACTIVE PERIOD: (photoplate -1)

Adrenal gland of the sexually inactive bat is oval, elongate to triangular in shape. The cotex shows three distinct zones viz. zona glomerulosa, zona fasciculata and Zona reticularis [Fig - 1].

Zona glomerulosa :

The outermost zone of the cortex, the zona gomerulosa consists of columns of cells having darkly stained nuclei each with a dark nucleolus. The cytoplasm of these cells stains faintly with basic dyes. Cytoplasm is eosinophilic and granular vaculations are observed in the cytoplasm of some cells. This is the smallest zone of adrenal cortex measuring 155µ during sexually inactive period. The zona glomerulosa imperceptibly merges with the zona fasciculata [Fig-2].

Zona fasciculata :

This is the largest cortical zone observed in the adrenal cortex measuring 1600µ during sexually inactive period which is lower than diameter of zona fasciculata of sexually active period. Zona fasciculata occurs in the form of cords of polyhedral cells, which are large and vacuolated. These cellular cords are long and are arranged radially. Binucleate cells are commonly noticed n the zona fasciculata. The cords of zona fasciculata are separated from each other by means of straight venous sinucoids. Each cell contain large nuclues with scattered chromatin clumps mostly near the peripheral region. Cytoplasm is eosinophilic and vacuolated due to the presence of lipid droplets. Zona fasciculata merges with zona glomerulosa above and zona reticularis below. Lipid vacuoles in cytoplasm which gives spongy appearance to the cytoplasm presence of cords and spongy cytoplasm are the charecteristics feature of this zone [Fig - 3].

Zona reticularis :

This zone is present just below the zona fasciculata and adjacent to medulla and measures about 340µ during sexually inactive period, which is lower than sexually active period. Zona reticulars consists of a network of cellular cords. The nuclei of some of the cells are pycnotic. Most of the cells contain a deposit of pigment. The shape and size of the cells vary. Each cell has eosinophilic cytoplasm with vesicular nucleus. Blood spaces are observed in the network of cell cords of zona reticularis [Fig - 4]

Medulla:

The medulla is distinctly demarcated from the cortex in *Pteropus giganteus giganteus*. It measures about 456 µ druing sexually inactive period. It is less extensive and the polymorphic epitheloid cells have a clear rounded nucleus with minute cytoplasmic granules. The cells of medulla are arranged in small groups or short cords surrounded by blood capillaries. Each cell has eosinophilic cytoplasm with vesicular nucleus [Fig-5].

[B] Adrenal gland during sexually active period (Photoplate-1)

During sexually active period it shows very typical and demarcable changes. Morphological studies reveal that, the size of adrenal gland during this period increases as compared to sexually inactive period. The shape also changes form oval to triangular during this period. Both Cortex and medulla are well developed and demonstrated separately [Fig - 1].

Zona glomerculosa :

Zona glomerulosa increase in size and measure about 190µ. The cells are elongated, arranged in columns with darkly stained nucleus, cytoplasm is eosinophilic and vaculations are observed in cytoplasm which are more than that observed in the cells of glomerulosa during the inactive period. [Fig- 2]

Zona fasciculata :

This is the widest cortical zone observed in the adrenal cortex measuring 1800µ during sexually active period. Zona fasciculata consits of large polyhedral or cuboidal cells arranged in cords. During this phase the hypertrophied cells of zona fasciculata show spongy cytoplasm with large number of lipid vacuoles, cytoplasm is eosinophilic. Nucleus is darkly stained. Zona fasciculata merges with zona glomerulosa above and zona reticularis below. Many sinusoids and blood capillaries are seen in the cell cords of this zone [Fig- 3].

Zona reticularis :

This zone is present just below the zona fasciculata and measures about 352 µ during sexually active period and is larger than the average diameter of zona reticularis of sexually inactive period. The nuclei of some cells are pycnotic while most of the cells of zona reticularis contains a deposit of pigment. The shape and size of cells vary and more vaculation are seen in the cytoplasm. Each cell has eosinophilic cytoplasm with vesicular nucleus. Blood spaces are observed in network of cell cords of this zone. [Fig-4]

Medulla:

Medulla is distinctly demarcated from cortex. Medullary zone of sexually active bat occupies largest area as compare to inactive bat. It measures about 468µ during this period. It consists of cells arranged in irregular strands or short cords surrounded by blood capillaries, and darkly stained vesicular nucleus. Cytoplasm is basophilic and granular. In some cells vaculations are observed [Fig-5].

DISCUSSION:

The size and weight of left adrenal gland is always higher than the right adrenal gland during different phases of reproductive cycle in the bat *Pteropus giganteus giaganteus*. Similar obervations are reported in *Hipposider lankadiva* [8] and *T. melanopogan*[13]. The adrenal gland weight is lowest during sexually inactive period, and highest druing sexually active period. Similar observation are reported in bat *T. melanopogan* [13] and *Herpestes auropunctatus*[4]. The medulla is clearly demarcated from the cortex of adrenal gland of bat *Pteropus giganteus giganteus*. Similar observations reported in the bat *Eptesicus*

fuscus[3] *M. lyra lyra*[6] *M.schereibersii*[1] and *R.leschenaulti*[8], *Cynopterous sphinx,* and *T. longimanous* [9].

The cortex is divided in three zones *viz.,* zona glomerulosa, zona fasciculata, and zona reticularis. This zonation of cortex is also observed in *M.lyra lyra* [6], *V. pipistrellus*[7], *M.scheribersis* [1]. Similar pattern of arrangement in zona fasciuata is oberserved in *M.lyra lyra* [6], *H. lankadiva* and *T. longimanus* [9], *R. leschenaulti* [8], *H. lankadiva* [8]. The adrenal gland of *Pteropus giganteus giganteus* is oval, elongate to triangular in shape and is enclosed in the conncective tissue capsule. Beneath the capsule large cortical zone divide distinctly into, zona glomerulosa, zona fasciculata and zona reticularis. The zona glomerulosa consist of columns of cells and similar structure is also reported in *R. leschenauti* [8], the cells are arranged in columns. The zona glomerulosa merges with zona fasciculata. The zona fascuculata occur in the form of cords of polyhedral cells which are large and vacuolated. These cellular cords are long, regular and are arranged radially. Similar pattern of arrangement in zona fasciuata is oberserved in *M.lyra lyra* [6], *H. lankadiva* and *T. longimanus* [9] The zona reticulars consists of a network of celluar cord of cells, which merge with zona fasciculata above and below with medulla. However, the zona reticularis is absent in *T. longimanus* [9]. The medulla of *Pteropus giganteus giganteus* shows well developed distinct, demarcations. Cells of medullar constist of numerous irregular strands or short cords of 4 to 5 cells surrounded by blood capillaries. Similar structure is reported in *M.lyra lyra* [6] *R.leschenauiti* [8] and *T.longimanus* [9]. Presence of two types of medullary cells represents the epinephrine and the norepinephrine secreting cells reported in *Rousettus leschenaulti,* on the basis of staining with basic types by Sapkal [8]

The size of adrenal gland during sexually active period is increase. It is oval, elongate to triangular in shape, during inactive period and changes oblong eliptical during active period. Histoarchitecture of adrenal gland druing sexually active period is advanced over that of adrenal gland of sexually inactive period. It shows significant difference at structural level. Zona fasciculata of sexullay active adrenal gland is well developed and made up of large polyhedral or cuboidal cells arranged in cords. Nucleus is darkly stained and spongy cytoplasm is seen with large number of lipid vaculoes, Thus zona faciculata is highly developed than other two zones.

Similar structures are also reported in *Hipposider lankadiva*[8] and *Taphonus melanapogaon* during active breeding period. The cells of glomerculosa are elongated, columns with darkly stained nucleus, Cytoplasm is eosinophilic and more vacuoles are observed in cytoplasm. During active period of reproductive cycle zona fasciculata is highly developed than the zona glomerulosa. Medulla of active period is well developed in the adrenal gland. Sesonal changes in adrenal gland in male bat *T.melanopogon* during reproductive cycle studies shows similar structures [13]. Hence adrenal gland of

these bats show significant differences of structure over arenal gland of inactive period bats, supporting present observations of *Pteropus giganteus giganteus* during inactive and active period.

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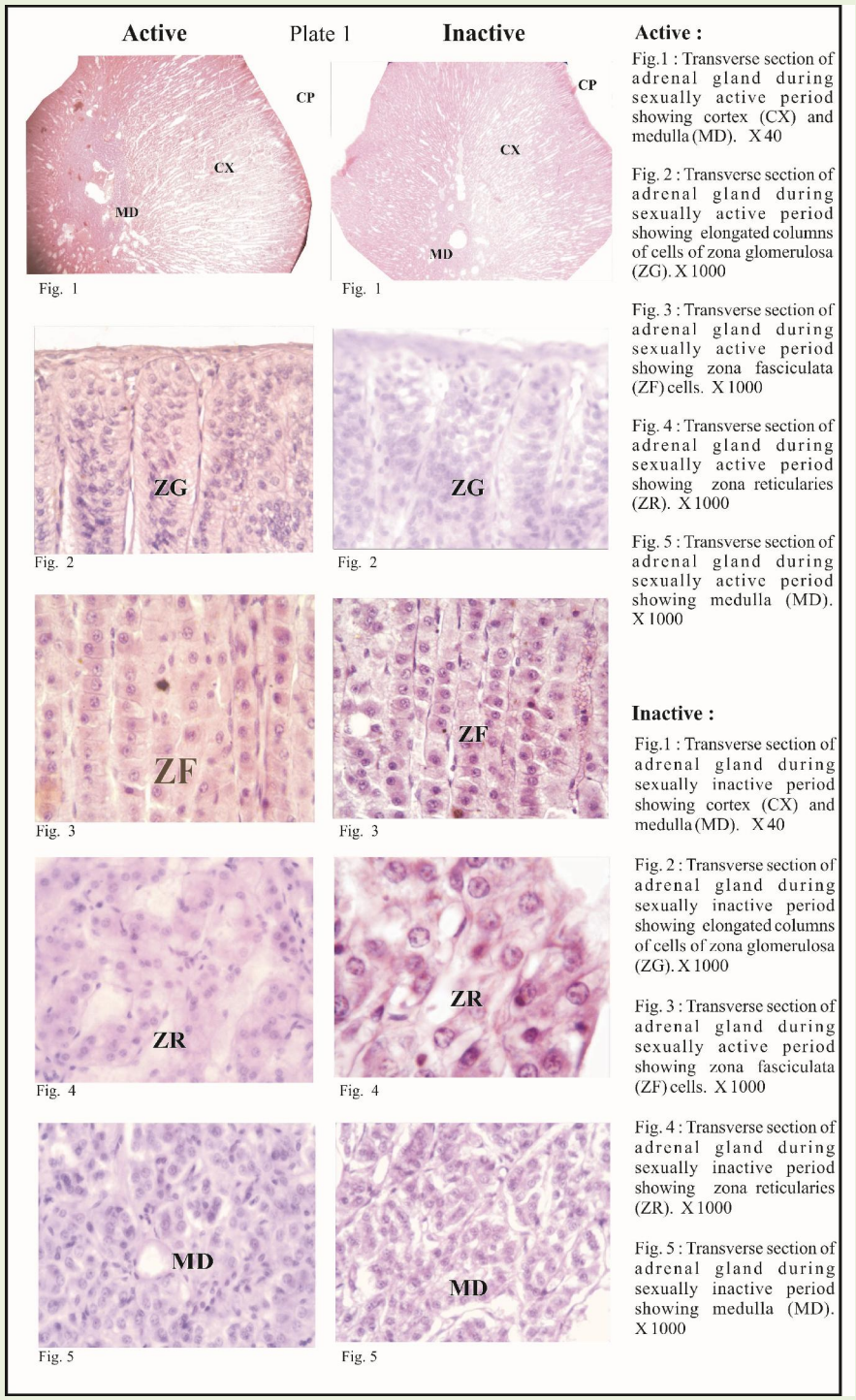

Active :

Fig.1 : Transverse section of adrenal gland during sexually active period showing cortex (CX) and medulla (MD). X 40

Fig. 2 : Transverse section of adrenal gland during sexually active period showing elongated columns of cells of zona glomerulosa (ZG). X 1000

Fig. 3 : Transverse section of adrenal gland during sexually active period showing zona fasciculata (ZF) cells. X 1000

Fig. 4 : Transverse section of adrenal gland during sexually active period showing zona reticularies (ZR). X 1000

Fig. 5 : Transverse section of adrenal gland during sexually active period showing medulla (MD). X 1000

Inactive :

Fig.1 : Transverse section of adrenal gland during sexually inactive period showing cortex (CX) and medulla (MD). X 40

Fig. 2 : Transverse section of adrenal gland during sexually inactive period showing elongated columns of cells of zona glomerulosa (ZG). X 1000

Fig. 3 : Transverse section of adrenal gland during sexually active period showing zona fasciculata (ZF) cells. X 1000

Fig. 4 : Transverse section of adrenal gland during sexually inactive period showing zona reticularies (ZR). X 1000

Fig. 5 : Transverse section of adrenal gland during sexually inactive period showing medulla (MD). X 1000