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Ultrastructural Characterization of Somatotrophs in Female Indian Flying Fox, *Pteropus giganteus giganteus* (Brünnich) During Reproductive Cycle

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Abstract: The aim of the present study was to reveal the ultrastructural changes in the somatotrophs (STH) during the reproductive cycle. Somatotrophs secrete growth hormones which stimulate growth and regulate metabolism. These cells are more in number as compared to the other glandular cells of pars distalis. STH cells are large, ovoid and have conspicuous plasma membrane. Cytoplasm is uniformly distributed throughout the cell. The secretory granules are large, round (200-400 nm in diameter) and electron-dense. During oestrous, pleomorphic hypertrophied mitochondria with lamellar cristae, well developed profiles of rough endoplasmic reticulum with elongated cisternae which are dispersed in the cytoplasm are noted. Golgi bodies are not distinct. Cytoplasm shows many electron dense secretory granules. During pregnancy STH cells are in an active state of secretion of growth hormone. Mitochondria are oval, few with collapsed or randomly distributed cristae. Golgi zone becomes prominent. Dilated profiles of rough endoplasmic reticulum are noted. However, the rough endoplasmic reticulum reduced in number during pregnancy. During lactation STH cells show a large number of oval electron dense secretory granules, most of them having diameter above 300 nm. Golgi bodies are also not distinct.

Keywords: Ultrastructure, Somatotrophs, Secretory granules, Pars distalis, Indian flying fox

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Introduction

Pituitary gland is the most important endocrine gland which regulates the reproductive physiology in mammals. Release of all hormones from the anterior pituitary gland are controlled by regulatory influence of the central nervous system i.e. hypothalamus and called as hypothalamic hormones transmitted to the anterior pituitary via the portal vessels. The growth hormone-releasing

hormone (GHRH) is a peptide hormone secreted by the hypothalamus that acts on somatotrophs and stimulates their proliferation and secretion of growth hormone (Mayo *et al.*, 2000). The secretion of growth hormones by the somatotrophs is due to the action of GHRH which causes the release of growth hormone from the secretory granules (Mayo *et al.*, 1995). Light microscopic and

ultrastructural characteristics of different cell types have been reported in various species of mammals during their reproductive cycle to understand the possible role of pituicytes in governing the physiological state of the animal (Kawamoto, 2003; Seraphim, 2004; Nerkar and Gadegone, 2010; Simbauni, 2012; Selim and El-Nahas, 2012). Selim and El-Nahas (2012) had studied the ultrastructure and histology of pars distalis of the Egyptian insectivorous bats *Taphozous nudiventris*. Nerkar and Gadegone (2010) had studied the ultrastructure of the pars distalis of the Indian female sheath-tailed bat, *Taphozous longimanus* (Hardwicke). They have identified the somatotrophs on the basis of shape and size of cell and nucleus, the size of secretory granules and activity of Golgi apparatus. The information about ultrastructural changes during the entire reproductive cycle in pituitary cell types are scanty. Thus the aim of present study was to investigate the ultrastructural changes in the somatotrophs during the reproductive cycle of female Indian flying fox *Pteropus giganteus giganteus*.

Materials and Methods

The Indian flying fox, *Pteropus giganteus giganteus* (Brünnich), a species of a fruit bat (Ali, 2022) which belongs to the Pteropodidae family of Megachiroptera suborder and Chiroptera order is selected for the present study. The study was conducted on the roosting colony inhabiting the *Ficus benghalensis* tree at Padmapur village in Chandrapur district in Vidarbha region at elevations of 20°11' N and 79°21' E. The specimens were obtained from natural populations. The specimens were collected in such a way that entire reproductive cycle was represented.

For transmission electron microscopy the tissues were fixed in 2.5 % glutaraldehyde and paraformaldehyde prepared in a cacodylate buffer (0.1 M, pH 7.4) and adjusted to pH 7.2 for 24 h at $\pm$ 4 °C. The tissues were rinsed in a cacodylate buffer and then post fixation was done for 3 h in 1% 0.067 M cacodylate-buffered osmium

tetroxide. Tissues were treated with a series of ethyl alcohol for dehydration. Dehydrated tissues were then cleared in propylene oxide solution. Then the sections were blocked in embedding molds with Araldite CY-212, dodecenyl succinic anhydride (DDSA) and benzyldimethylamine (BDMA) which were polymerized at 60 °C. Semithin sections were visualized under microscope after staining with toluidine blue. Then, ultrathin sections from selected blocks were cut with a glass knife (300 –700 Å thick) and were double stained with 10% alcoholic uranyl acetate for 20 min and in Reynold's lead citrate for 10 min. Sections were picked up on 400-mesh copper grids. The sections were examined and Electron micrographs were taken by electron microscope (JEOL-100 S) at 80 KV accelerating voltage.

Results

In the anterior pituitary of *Pteropus giganteus giganteus*, six cell types have been noted based on the shape, size and position of the cytoplasmic secretory granules and nucleus present in the pituicytes. The size of the secretory granules, the activity of Golgi apparatus, and changes in endoplasmic reticulum were considered to determine the secretory state of the cells. Two types of acidophilic cells, three types of basophilic cells and chromophobes have been observed by ultrastructural characterization. Among acidophils somatotrophs and mammotrophs which produce growth hormone and prolactin respectively have been identified. However, basophils constitute three cell types, that are corticotrophs, thyrotrophs and gonadotrophs. Corticotrophs secretes adrenocorticotropin and thyrotrophs secretes thyroid stimulating hormone whereas gonadotrophs produce luteinizing hormone and follicle stimulating hormone. Sixth cell type the chromophobes, is a folliculostellate cell devoid of secretory granules.

Somatotrophs:

These are the most abundant cells in the adenohypophysis specially in the pars distalis region. They are large, ovoid and found in groups

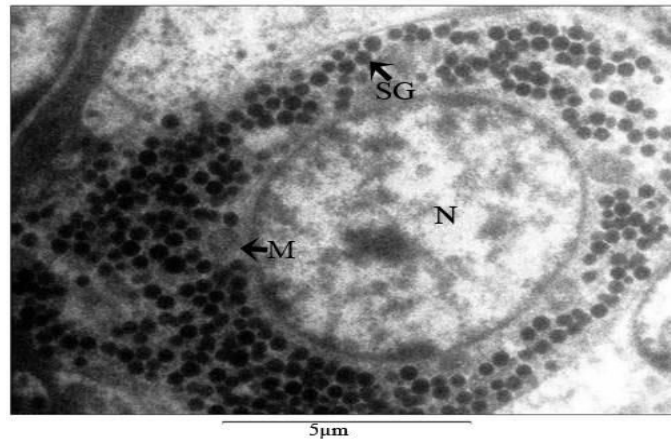


Fig. 1: Electron photomicrograph of the STH cell in oestrous female. Note the presence of an eccentric nucleus (N), oval to elongated mitochondria (M) with lamellar cristae, and the presence of a large number of secretory granules (SG). (2000X)

around capillaries and sinusoids. These cells have conspicuous plasma membrane. Cytoplasm is uniformly distributed throughout the cell. The secretory granules are large, round (200-400 nm in diameter) and electron-dense.

Somatotrophs during oestrous:

During oestrous, somatotrophs are oval to spherical in shape. Nucleus is oval in shape and eccentric in position. The nucleus is euchromatic and clumps of chromatin material are noted at the peripheral region of the nucleus. Nucleus shows the nuclear pore, however, nucleolus is not conspicuous. The presence of pleomorphic hypertrophied mitochondria with lamellar cristae, well developed profiles of rough endoplasmic reticulum with elongated cisternae is dispersed in the cytoplasm. Free ribosomes are seen scattered throughout the cytoplasm. Golgi bodies are not distinct. Large number of oval osmiophilic secretory granules (200-400 nm) of varying size and vacuolated dense bodies are present in the cytoplasm. Cytoplasm shows many electron dense secretory granules. Thus the cytoplasm of these cells is electron dense in nature. Few lipid droplets are also noted (Figs. 1, 2).

Somatotrophs during pregnancy:

At the onset of pregnancy, ultrastructure of somatotrophs shows significant variation. Mitochondria are oval with few collapsed or randomly distributed cristae. Golgi zone becomes prominent. Dilated profiles of rough endoplasmic reticulum are noted. However rough endoplasmic reticulum reduced in number during pregnancy (Figs. 3, 4).

Somatotrophs during lactation:

During lactation somatotrophs show a large number of oval electron dense secretory granules. All the secretory granules are of varying diameter but variation is not of wide range. Most of the secretory granules have a diameter above 300 nm. Nucleus is euchromatic with chromatin material dispersed at the periphery of the nucleus. Nuclear pore is more conspicuous. Nucleolus is not distinct. Very few rough endoplasmic reticulum and mitochondria are noted. Similarly Golgi bodies are also not distinct. Free ribosomes are seen scattered throughout the cytoplasm (Figs. 5, 6).

Discussion

Ultrastructural studies on adenohypophysis in *Pteropus giganteus giganteus* reveals the presence of six cell types in oestrous, pregnant and lactating females, which shows a variation in cytological

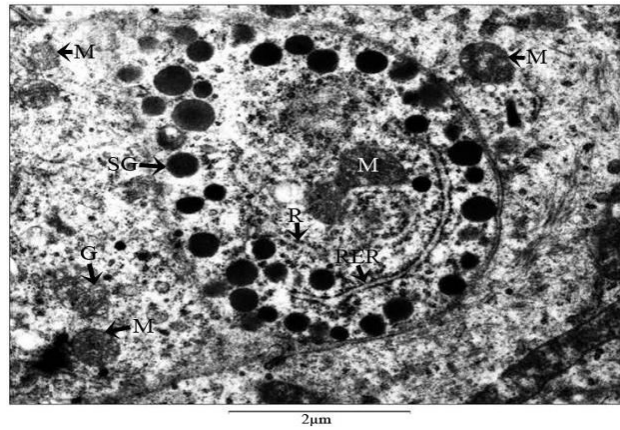


Fig. 2: Electron photomicrograph of the STH cell in oestrous female. Note the presence of pleomorphic mitochondria (M) with lamellar cristae, elongated dilated profiles of RER dispersed in the cytoplasm. Presence of a large number of secretory granules (SG) and vacuolated dense bodies. (4000X)

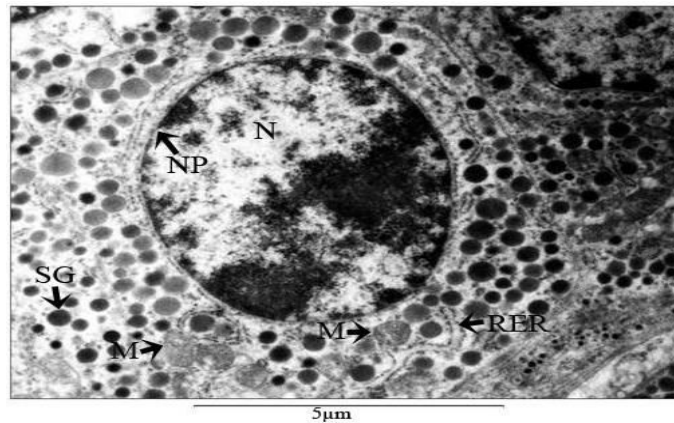


Fig. 3: Electron photomicrograph of the STH cell in pregnant female. Note the presence of nucleus (N) with chromatin clumps, oval mitochondria (M) with lamellar cristae, elongated profiles of RER dispersed in the cytoplasm and large number of secretory granules (SG). (2500X)

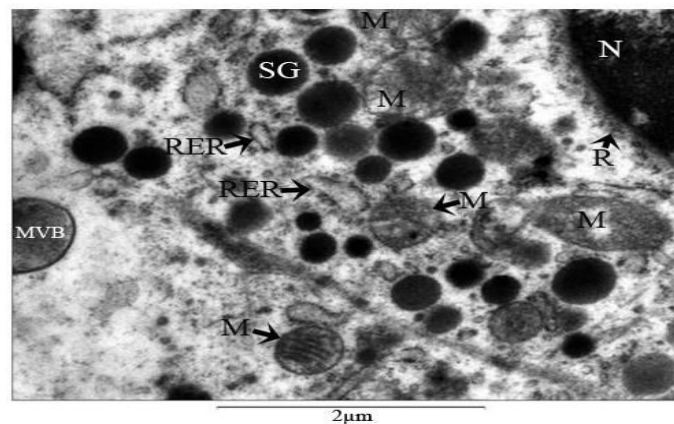


Fig. 4: Electron photomicrograph of the STH cell in pregnant female. Note the presence of nucleus (N) with chromatin clumps at the periphery, oval to elongated mitochondria (M) with lamellar cristae, elongated profiles of RER dispersed in the cytoplasm and large number of secretory granules (SG) of varying diameter. (2500X)

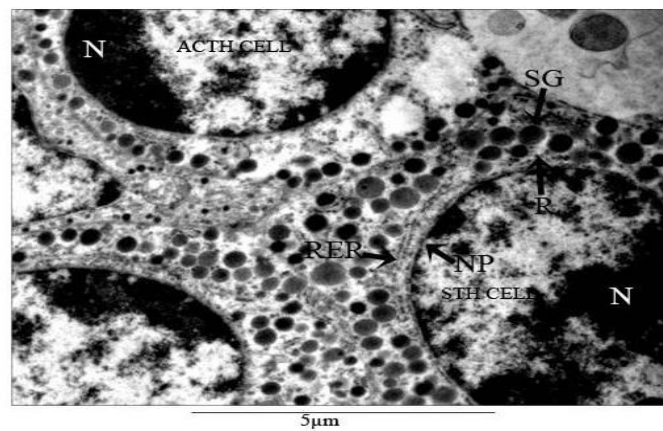


Fig. 5: Electron photomicrograph of pars distalis in lactating female. Note the STH and ACTH cell with clumps of chromatin material at periphery, elongated profiles of RER dispersed in the cytoplasm and secretory granules (SG). (2500X)

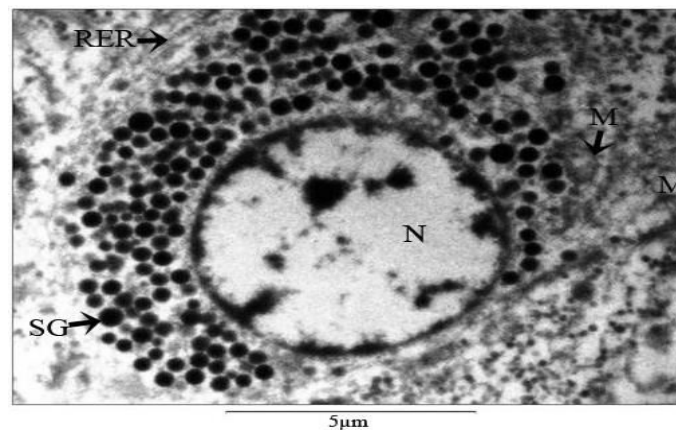


Fig. 6: Electron photomicrograph of the STH cell in Lactating female. Note the presence of nucleus (N) with chromatin clumps at the periphery, oval to elongated mitochondria (M) with lamellar cristae, elongated profiles of RER dispersed in the cytoplasm, presence of large number of electron dense secretory granules (SG). (2500X)

characters with the reproductive cycle as in other bats. Herlant (1964) in *Myotis myotis*, Azzali (1971) in *Vesperugo savi* and *Vesperugo piccolo*, Richardson (1979) in *Macrotus californicus*, Mikami *et al.* (1988) in *Miniopterus schreibersii fuliginous*, Bhiwgade *et al.* (1989) in *Rousettus leschenaulti*, Singh and Krishna (1994) in *Scotophilus heathi*, Seraphim (2004) in *Hipposideros lankadiva*, Simbauni (2012) in *Eidolon helvum*, and Nerkar and Gadegone (2010) in *Taphozous longimanus* have observed six cell types in anterior pituitary which is similar to that found in *Pteropus giganteus giganteus* supporting present observations.

STH cells are the most abundant cells in the adenohypophysis specially in the pars distalis region. These cells are oval to spherical in shape having eccentrically placed oval nucleus and characterized by uniformly distributed cytoplasm throughout the cell and large electron-dense secretory granules measuring 200-400 nm in diameter. No marked variation in shape and size during different phases of sex cycle. State of Golgi bodies depends on secretory activity of the cell. Golgi bodies are not distinct during oestrous and lactation, however, prominent Golgi zones are observed during pregnancy. Somatotrophs filled with a large number of secretory granules indicate

the storage state of the cell whereas presence of a small number of secretory granules indicates the synthetic activity of the cell. The presence of pleomorphic hypertrophied mitochondria with lamellar cristae, well developed profiles of rough endoplasmic reticulum with elongated cisternae is dispersed in the cytoplasm.

Bhiwgade *et al.* ((1989) had studied the ultrastructural characteristics of STH cells of Indian fruit bat, *R. leschenaulti* and noted that these cells are round to oval in shape with centrally placed round nucleus. The Golgi apparatus is inconspicuous and mitochondria are round and scattered in the cytoplasm. The secretory granules are numerous, uniform, round and very dense about 350-400 nm in diameter.

In *Scotophilus heathi*, Singh and Krishna (1994) have observed the STH cells having round mitochondria and Golgi bodies. Electron dense secretory granules of 350-400 nm in diameter are noted.

Seraphim (2004) observed the somatotrophs in *Hipposideros lankadiva* which are round to oval with eccentrically placed nuclei. The secretory granules are numerous, mostly round to oval with uniform electron density. The well developed Golgi showed the synthetic and storage state of cells. Almost similar type of ultrastructure is noted in cow (Mikami, 1970) and Mink (Weman, 1974) supporting the present observations in female *Pteropus giganteus giganteus*.

Conclusion

During present investigation STH cells are the most abundant cells in the adenohypophysis specially in the pars distalis region of female *Pteropus giganteus giganteus*. The somatotrophic cells observed were in an active state of secretion of growth hormone during pregnancy having a prominent Golgi zone indicating synthetic activity of the cell. However, these STH cells were filled with a large number of secretory granules during oestrous and lactation indicating the storage state of the cells. No marked variations were noted in shape and size of the STH cells.

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