Journal of Microscopy and Ultrastructure 4 (2016) 108–114

Contents lists available at ScienceDirect

Journal of Microscopy and Ultrastructure

journal homepage: www.elsevier.com/locate/jmau

Original Article

Ultrastructural characterization of Gonadotrophs in the Wild caught female bat *Taphozous nudiventris kachhensis* (Dobson)

Pankaj R. Chavhan[a,*], Amir Dhamani[b]

[a] *Shri Sadguru Saibaba Science College, Ashti, 442707 India*
[b] *Gram Geeta College, Chimur, India*

ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form 23 October 2015
Accepted 5 November 2015
Available online 23 November 2015

Keywords:
Pituitary gland
LH
FSH
Bat

ABSTRACT

Present study has been design to observe the ultramicroscopic structure of Gonadotrophs in the female bat *Taphozous nudiventris kachhensis* during the various phases of reproductive cycle. During the present study specimen were collected during Estrous and pregnancy to know the probable role of Gonadotrophs (FSH, LH) in respective stage of reproductive cycle. After collecting female specimen they were weight and dissect out for the pituitary gland and then those were fixed in ice cold gluteraldehyde. Gonadotrophs (FSH and LH) are mostly frequently observed cell types after Somatotrophs cells in the pars distalis of the female bat *Taphozous nudiventris kachhensis*. FSH cell during estrus is large, ovoid to polyhedral in shape with irregular shaped nucleus. Cell cytoplasm shows well developed Golgi apparatus, rough endoplasmic reticulum and mitochondria this indicating active state. During early pregnancy cytoplasm of FSH cell appears vacuolated because of dilation of rough endoplasmic reticulum. Mitochondria are spherical to rod shaped with lamellar cristae. During late pregnancy rough endoplasmic reticulum shows dilation. Golgi complex is juxtanuclear in position and mitochondria are numerous. LH cells during early part of gestation are large with bilobed nucleus. Cell cytoplasm shows well developed rough endoplasmic reticulum heavily dotted with ribosomes and contains large number of secretory granules. Hypertrophied LH cell in pars distalis of bat during late pregnancy shows well developed rough endoplasmic reticulum occupies major part of cytoplasm. Golgi zone is well developed with dilated saccules. Secretory granules are very few.

1. Introduction

In India this insectivorous bat is a seasonal breeder and bread once in a year. We collect sample during this period. Reproductive and life-history strategies vary tremendously among bats, even within species. Understanding the nature of this variation and the evolution of these strategies requires an understanding of the mechanisms responsible. The pituitary cells are regulated by numerous endocrine, paracrine and autocrine feed-back pathways, and their hormone secretion exerts major control over the function of several endocrine glands as well as a wide range of physiologic states. Endocrine control of reproduction in bats is reviewed by Anthony [2]. The role of gonadal hormones in the reproduction of the bats is reviewed by Martin and Bernard [15] and role of peripheral endocrine organ in reproduction of bats has been reviewed by Kwiencinski and Damassa [14].

The ultrastructural study of the pars distalis has been mainly investigated in laboratory mammals, with the goal of defining not only microscopic characteristics but also the physiological significance of different cell categories

* Corresponding author.
E-mail address: panksphd@gmail.com (P.R. Chavhan).

http://dx.doi.org/10.1016/j.jmau.2015.11.001
2213-879X/

with respect to reproduction. The influence of pituitary hormones, such as LH and FSH are fundamental to reproductive physiology. These cells of pars distalis not only play a pivotal role in reproductive process of mammals but also show changing morphology during different phases of reproductive cycle of bats [4,5,9,24,26,23,28].

Herlant [8], Peyre and Herlant [25], Badwaik [4] and O'Brien et. al [24] have reported that these cell have different morphological feature at different reproductive phases. In the present study ultrastructure of FSH and LH are described with reference to Golgi body, Endoplasmic reticulum, secretory vesicles, mitochondria and nucleus. This study is paid attention because there is very little information available on structure of pituitary gland at ultrastructural level in Chiroptera (bats). The information and data provided in this study will throw a light on the understanding of reproductive behavior of bats and endocrinology of mammals. Furthermore, bats are nocturnal and their reproductive strategies somewhat differ with other mammals. Therefore, anatomical studies of bats like this study are so important.

2. Material and Methods

Taphozous nudiventris kachhensis (Dobson) is an exclusive Indian Emballonurid bat found in caves, tunnels and temples. The gestation length of female of this species is about 150 days. The collection of the specimen commenced in February 2006 and the last specimen for the present study was collected in May 2009. The specimens of *Taphozous nudiventris kachhensis* were collected from Ambai Nimbi, about 45 kilometers from Bramhapuri Taluka, District, Chandrapur, Maharashtra, India. Many collections were made during the breeding season so as to coincide with the time of reproductive cycle and to get an accurate pregnancy record. During the day time, their roosting places were visited and the specimens were netted at random with the help of a butterfly net. During each collection we collect 5 specimens and after observing mammary glands and pelvic dugs 1 Mature female is separated and rest were released. The specimen is killed by decapitation and pituitary gland is fixed for Transmission electron microscopy.

2.1. Transmission electron microscopy

Pituitary glands of the species from pregnant and non-pregnant specimen were selected for electron microscopic study.

2.1.1. Fixation

Pituitary gland is removed from the bat and cut into 1-2 mm piece and immersed in fresh ice-cold 3% gluteraldehyde solution. The fixation was carried out over a period of 1 to 2 hr at 4^0c.A fresh change of cold gluteraldehyde was given at the end of fixation and the tissue were washed in cold 0.1 M sodium cocodylate buffer for half an hour with 3 to 4 changes to ensure complete removal of excess gluteraldehyde. Post fixation with OSO_4 or osmification with 1% OSO_4 in sodium cocodylate buffer was carried out for 2 hr at 4^0c.

2.1.2. Dehydration

Dehydration of tissue was carried out by passing the fixed tissues through a graded series of alcohol of increasing concentration of the dehydrating agent in water ending with absolute alcohol. Most epoxy resins are soluble in ethyl alcohol and acetone but they mix much in propylene oxide. Thus tissues were passed through intermediate solvent, propylene oxide over a period of half hour.

2.1.3. Infiltration and embedding

Complete and uniform penetration of tissue by the embedding medium is accomplished through infiltration and embedding. Infiltration involved the gradual replacement of dehydrating agent with embedding medium while embedding consist of complete impregnation of the interstices of a tissue specimen with the medium. This was done as follows:-

i) Propylene oxide araldite 'A' solution 1:1 for one hour at room temperature.
ii) Fresh araldite 'A' solution–kept at room temperature in desiccator overnight.
iii) Araldite 'B'solution–for 1 hour at room temperature.

Embedding of tissue was done in plastic BEEM capsule with fresh araldite 'B' solution and the capsule was kept in an oven maintained at 60^0 C for 24-48 hours to ensure polymerization. Blocks were freed from the sample by cutting away the plastic, then trimmed with safety razor blade under a stereo-microscope, to a flat surface cone, to remove the excess embedding material ultrathin section of 1-2 micron in thickness were cut on an LKB ultratome V, with glass knife maker. These sections were dried on hot plate (60^0 C) and consequently stained with 1% toludine blue (20-30 seconds) and observed on light microscope. The selected areas for ultrathin section were marked out. The blocks were further trimmed and ultrathin section were marked out the block were further trimmed and ultrathin section or thin sections'600-900 A^0 thick corresponding the pale gold colour of section were cut section were collected on 300 mesh copper grids. To enhance the contrast double staining technique was employed. The grid was subjected to 10% alcoholic uranyl acetate for half an hour followed by lead citrate for 10 minutes. All grids were observed on a JEOL-100S electron microscope at 80 KV accelerating voltage. Micrographs were taken of the desired sample at different planes.

3. Result and discussion

In *Taphozous nudiventris kachhensis* the gland is dorsoventrally compressed and semicircular in shape. Gonadotrophins (FSH and LH) are mostly frequently observed cell types after Somatotrophs in the pars distalis of the female bat *Taphozous nudiventris kachhensis*. These cells are located near the blood capillaries. In the present study the identification and characterization of the FSH and LH cells is based on the morphological features of the cellular constituents such as secretory granules, ergastoplasm, Golgi apparatus and Mitochondria.

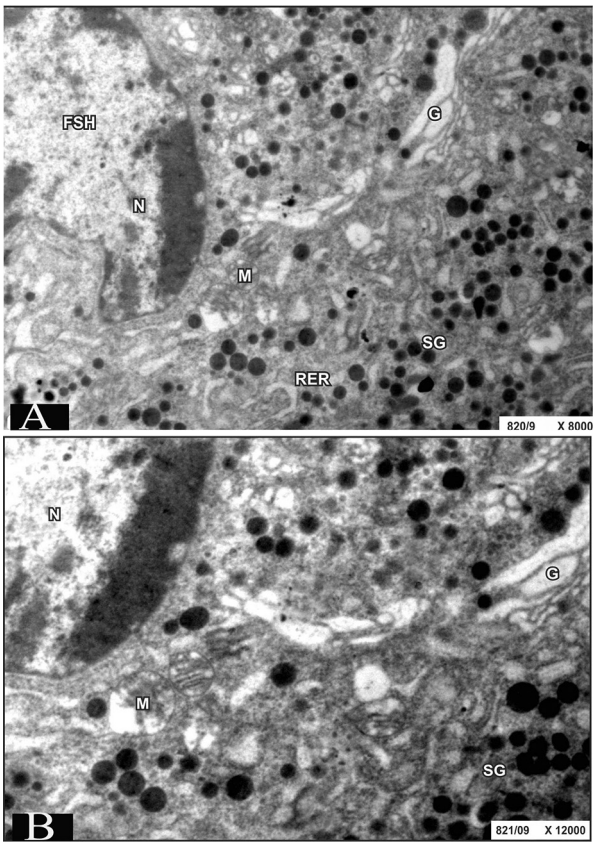

Fig. 1. (A) Electronmicrograph of the FSH in the pars distalis during estrus. Note the presence of indented nucleus [N] with irregular outline **X 8000. (B)** Magnified view of the FSH cell during estrus showing hypertrophied Golgi apparatus [G] consist of dilated saccules and small vesicle, hypertrophied mitochondria [M] with collapsed cristae and few mitochondria with lamellar cristae. Note the presence of tubular cisternae of rough endoplasmic reticulum [RER] and secretory granules [SG] are distributed throughout the cytoplasm. **X 12000**.

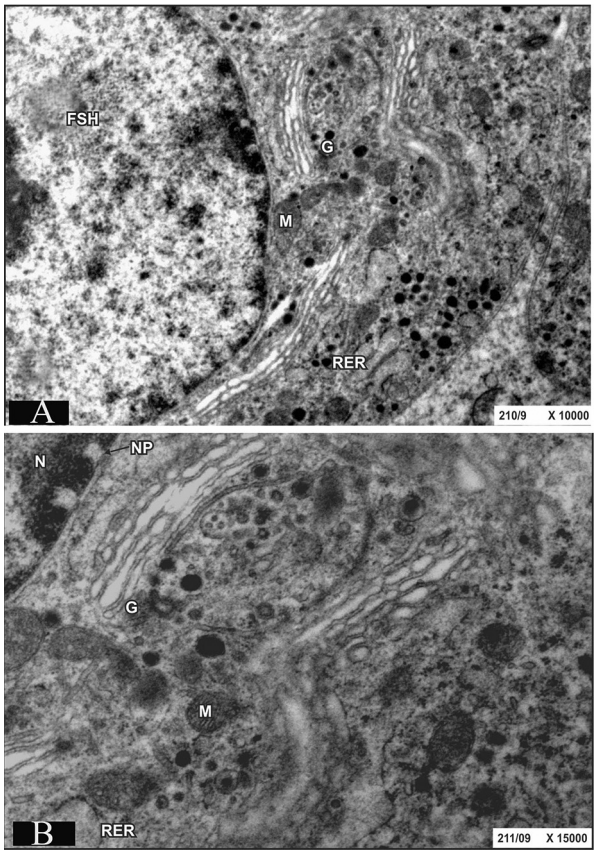

Fig. 2. (A) Electronmicrograph of the FSH in the pars distalis during early pregnancy. Note the presence of oval shaped nucleus [N] with thin rim of chromatin material at periphery, well developed Golgi apparatus [G], dilated cisternae of rough endoplasmic reticulum [RER], mitochondria [M] with collapsed cristae and few secretory granules [SG]. X10000. **(B)**Magnified view of the FSH cell during early pregnancy shows highly developed Golgi apparatus [G]. Note the presence of spherical mitochondria [M] with lamellar cristae. Free ribosomes are seen scattered in the cytoplasm. Note the presence of dilated cisternae of endoplasmic reticulum [ER]. **X 15000**.

3.1. FSH CELL

3.1.1. FSH Cell during Estrus

FSH cells during estrus showed pronounced changes under electron microscopy. The cells are large, ovoid to polyhedral in shape with irregular nucleus and shows indentation. Heterochromatin flakes are seen scattered throughout the nucleoplasm. The nuclear pores are clearly visible. Golgi apparatus is hypertrophied and juxtanuclear in position. The outer zone has slightly dilated saccules while the maturing face shows several associated vesicle, vacuoles and newly synthesized secretory granules. Mitochondria are spherical, with randomly spread lamellar cristae and present in the Golgi zone. Several elongated profiles of Rough endoplasmic reticulum are observed throughout the cytoplasm. Some of these have dilated cisternae of varying degree. These are heavily dotted with ribosomes (Fig. 1 A & B).

3.1.2. FSH cell during early pregnancy

The nucleus of FSH cell is slightly indented with clumping of chromatin material and one or two nucleoli at the center (Fig. 2 A). Mitochondria are dispersed in the cytoplasm; they are small and can be round or elongated with vesicular cristae and some with lamellar. Rough endoplasmic reticulum is highly developed and cisternae are greatly dilated. Golgi apparatus consist of saccules and vesicle arranged in circular array. In Golgi area immature granules are seen. Dilated large saccules of Golgi enclosed only some newly synthesize secretory granules toward their maturing face. Secretory granules are small in size and scattered throughout the cytoplasm (Fig. 2 B).

3.1.3. FSH cell during mid pregnancy

As the pregnancy advanced cytoplasm appears vacuolated due to the dilation of rough endoplasmic reticulum. (Fig. 3 A). These dilated cisternae are sparsely dotted with ribosomes and distributed throughout the cytoplasm. The lumina of the cisternae are filled with less osmophilic material (Fig. 3 B). Mitochondria are hypertrophied with collapsed cristae and some with lamellar cristae. Golgi apparatus is indistinct.

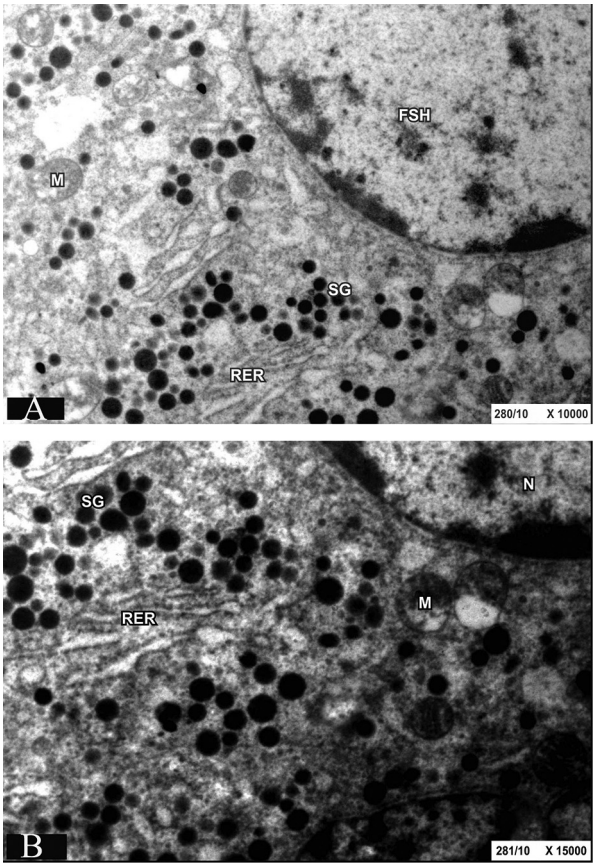

Fig. 3. (A) Electronmicrograph of the FSH cell in the pars distalis during mid pregnancy. Note the presence of nucleus [N] with flakes of chromatin material distributed in the nucleoplasm. X 10000. **(B)** Magnified view of the FSH cell during mid pregnancy shows hypertrophied mitochondria [M], parallely arranged array of rough endoplasmic reticulum [RER]. Note the presence of secretory granules [SG]. X 15000.

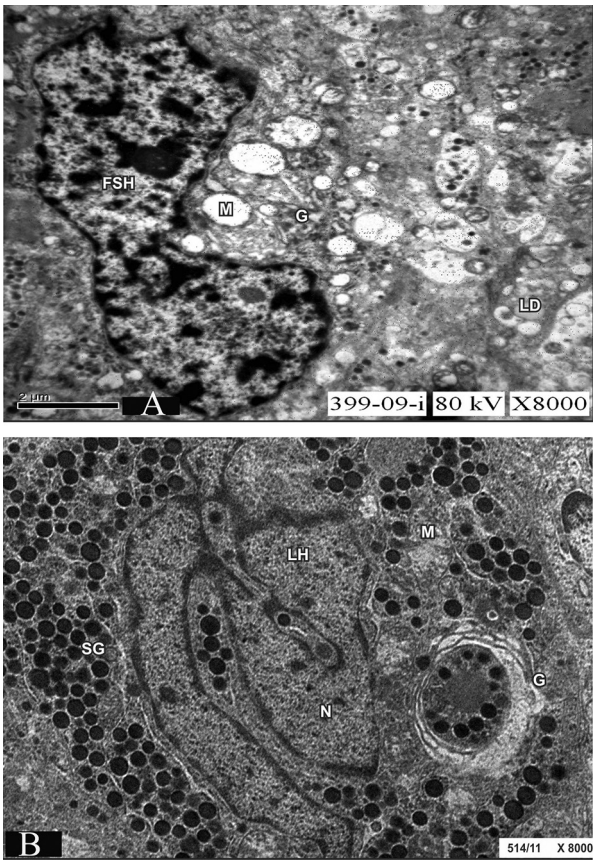

Fig. 4. (A) Electronmicrograph of the FSH cell in the pars distalis during late pregnancy. Note the presence of indented nucleus with flakes of heterochromatin material scattered in the nucleoplasm with well developed nucleolus [NO]. Note the presence of spherical mitochondria. [M] with collapsed cristae. Golgi apparatus [G] is well developed. Few lipid droplets [LD] are seen. **X 8000. (B)** Electronmicrograph of the LH in the pars distalis during early pregnancy. Note the presence of large, bilobed nucleus [N], mitochondria with lamellar cristae and secretory granules [SG] of variable electerondensity. Note the presence of Golgi apparatus [G] arranged in semicircle. **X 8000**

Secretory granules are seen scattered in the cytoplasm and more toward the apical cytoplasm.

3.1.4. FSH cell during late pregnancy

There is further hypertrophy of FSH cells during late pregnancy. Nucleus is irregular in outline and show many indentation. Nucleolus is prominent. In contrast to FSH during estrus bat FSH cell contain very few secretory granules and lysosome seen in the cytoplasm. Mitochondria are numerous, with collapsed cristae (Fig. 4 A). Golgi apparatus is inconspicuous. Secretory granules are very few and scattered throughout the cytoplasm. FSH cells shows well developed cell organelles such as Mitochondria, Golgi body, and rough endoplasmic reticulum during pregnancy and elaborate large amount of follicle stimulating hormone.

3.2. LH CELL

3.2.1. LH during early pregnancy

During early pregnancy LH cell are large with bilobed nucleus. Chromatin material is attached to inner part of the nuclear membrane (Fig. 4 B). Mitochondria are elongated in shape and hypertrophied with collapsed cristae. Rough endoplasmic reticulum is dilated and studded with ribosomes. Golgi apparatus is juxta nuclear in position. Golgi complex is made up of 3 to 4 golgi saccules. Small granules are observed in the golgi region. Golgi saccules are curved and dilated at the ends. Large numbers of secretory granules of varying electron density are observed throughout the cytoplasm (Fig. 5 A).

3.2.2. LH during mid pregnancy

Hypertrophied LH Gonadotrophins cells in the pars distalis of pregnant bat during mid pregnancy show well developed smooth endoplasmic reticulum. The mitochondria are spherical with lamellar cristae. Golgi saccules are made up of 2to3 Golgi saccules and are curved (Fig. 5 B). Flatten stakes of rough endoplasmic reticulum are dotted with ribosomes and are distributed throughout the cytoplasm. Free ribosomes are large in number and observed in the cytoplasm. Secretory granules are small in size and more toward the apical region.

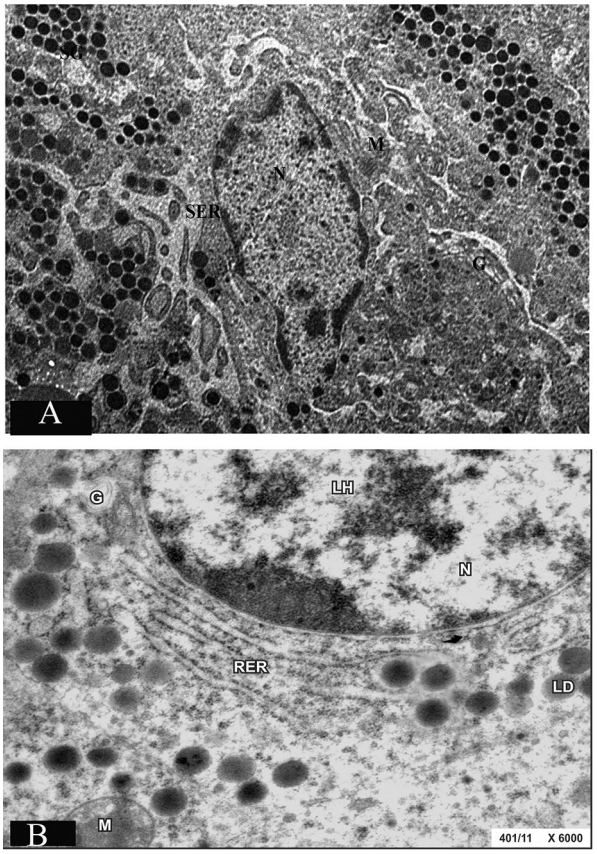

Fig. 5. (A) Electronmicrograph of the LH in the pars distalis during mid pregnancy showing indented nucleus [N]. Note the presence of hypertrophied Golgi apparatus [G]. Smooth endoplasmic reticulum [SER], hypertrophied mitochondria [M]. Secretory granules [SG] distributed toward the periphery of the cell. **X 8000. (B)** Electronmicrograph of the LH in the pars distalis during late pregnancy showing irregularly shaped nucleus [N], dilated cisternae of rough endoplasmic reticulum [RER]. Note the presence of circular mitochondria [M] with lamellar cristae and osmophilic lipid droplets [LD]. **X 6000**.

3.2.3. LH during late pregnancy

The LH cells during late pregnancy are hypertrophied and show well developed rough endoplasmic reticulum and occupies more part of cytoplasm. LH cell are large. The cisternae of rough endoplasmic reticulum are arranged in parallel array. Some flattened cisternae and vesicle of rough endoplasmic reticulum are observed in the cytoplasm. Golgi zone is well developed with dilated saccules at the forming face and flattened saccules toward the maturing face (Fig. 5 B). Secretory granules are larger in size but few in number as compared to previous phase. (Fig. 6A). LH cell shows marked development of cell organelles such as Mitochondria, Golgi body, Rough endoplasmic reticulum, thus these cells are very active and elaborate large amount of luteinizing hormone.

4. Discussion

The present study demonstrates that the two types of Gonadotrophs in the anterior pituitary which undergoes number of changes in cytological character with

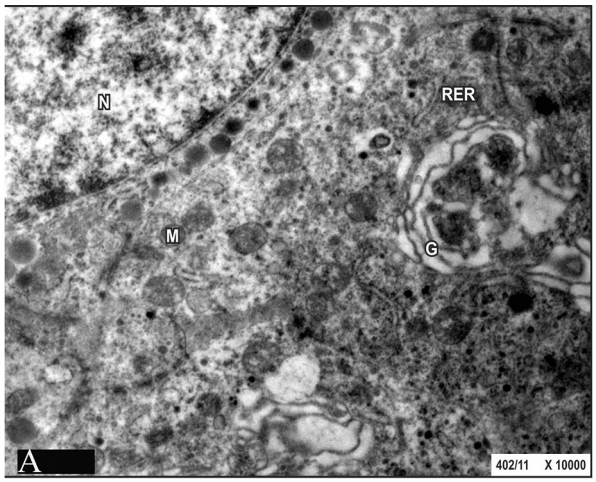

Fig. 6. (A) Magnified view of the LH cell in the pars distalis during late pregnancy showing dilated Golgi apparatus [G] near the nucleus [N]. Note the presence of polymorphic profiles of rough endoplasmic reticulum [RER]. Note the arrangement of secretory granules [SG] near the nuclear membrane. **X 10000**

reproductive state. In the present study *Taphozous nudiventris kachhensis* is a seasonal breeder and cycle ranges from October to July, while in Egyptian bat *Taphozous nudiventris* [1] breeding cycle ranges from March to July which is differ from our observations.

Much of the information gathered from various work on the pituitary gland indicates that glandular cells of the pars distalis once differentiated produce only one hormone [32]. With the advent of immunocytochemical procedure the 'one cell, one hormone' hypothesis was contested by some worker [8,13,17,18,19,21] who on the basis of immunocytochemical studies on these cells indicate that more than one hormone was produced by the same cell.

Morphological studies at electron-microscopic level continue to extend our knowledge of the pituitary's functional organization. Azzali [3] studied the cytology and physiology of the adenohypophysis of *Vesperugo savi* and *Vesperugo piccolo* and identified six types of secretory cells and a seventh type without secretory granules. Bhiwgade et al. [5] studied the ultrastructural and functional characteristics of the anterior pituitary cells in the Indian fruit bat, *Rousettus leschenaulti*. Ultrastructural changes in gonadotrophic and prolactin cells of *Myotis myotis* under experimental conditions have been studied by Muniz et al. [18].

The influence of pituitary hormones, such as, LH, FSH and prolactin are fundamental to reproductive physiology. These cells of pars distalis not only play a pivotal role in reproductive processes of mammals but also show changing morphology during different phases of reproductive cycle of bats [4,5,12,25].

Several researchers by electron microscopy have reported [6,7,27] that morphological differences between FSH and LH producing cells exist. Herlant, [8–11,14,20] and Azzali, [3] studied cytological variations in gonadotrophs in different species of bats under various physiological conditions. They have differentiated the gonadotrophs into two distinct entities, the FSH and LH secreting gonadotrophs.

According to Bhiwgade et al. [5] the variations observed in the electron density of the secretory granules is sufficient to differentiate two types of gonadotrophs. These cells also differ in their cytoplasmic organelles and do not show similar changes under altered conditions such as estrus, pregnancy and lactation.

Ultrastructural features observed in *Taphozous longimanus* [22] FSH cells in the pars distalis of estrus female are large ovoid to polyhedral in shape. Golgi apparatus is well developed. Mitochondria are hypertrophied with collapsed cristae. Rough endoplasmic reticulum is well developed. Secretory granules are spherical, 200-400 nm in diameter and show variable electron density. These ultrastructural features suggest that the FSH cells are metabolically active during estrus. During pregnancy, FSH cells undergo hypertrophy. Nucleus is irregular in outline. Large numbers of mitochondria with collapsed cristae are present in the cytoplasm. The dilated cisternae of rough endoplasmic reticulum are sparsely dotted with ribosome and are distributed throughout the cytoplasm and thus the cytoplasm appears vacuolated. A few electron dense granules are seen more towards the apical part of the cytoplasm. The hypertrophy of FSH cells during late pregnancy is associated with filigreed cytoplasmic pattern giving a bizarre appearance, because of further dilation of cisternae of endoplasmic reticulum.

Ultrastructural characteristics of FSH cells of *Taphozous nudiventris kachhensis* are similar to that reported in other bat species [19,3,29,30,32,22,23]. Muniz et al. [18] studied ultrastructural characteristics of gonadotrophs of pregnant bat, *M. myotis.* The granules of the gonadotrophic cells are columnar or cuboidal and are medium electron density. The gonadotrophs of pregnant bat under experimental conditions show degranulation which display greatly increased development of the rough endoplasmic reticulum and Golgi apparatus, a large number of lysosomes and a large amount of degraded material. Azzali, [3] observed cytological changes in the LH cells of *V. savi* and *V. piccolo* with electron microscopy and suggest that delayed ovulation may be the result from insufficient quantities of LH and that the Graafian follicle is maintained by tonic secretion of FSH.

In the present study, ultrastructural features of FSH cells indicate that they are synthetically very active during estrus and pregnancy when ovary shows folliculogenesis and a Graafian follicle. Our observations suggest that the hypertrophied FSH cells at late pregnancy are necessary for maintaining Graafian follicle in the contra lateral ovary, which is going to ovulate.

In *Megaderma lyra lyra* [31] the gonadotrophs during estrous period are angular with irregular nucleus. The secretory granules are electron dense and of large size. Few lysosome are observed. The rough endoplasmic reticulum is in the form of vesicles. Golgi is very well developed. While mitochondria are circular with lamellar cristae.

The observation s made on LH cells in *Taphozous nudiventris kachhensis* were Similar in LH cell by Herlant [11]. It also corresponding with LH cell in *Miniopterus schreibersii fuliginosus* during estrus, described by Mikami et al. [16]. The volume of LH cell during estrus is well needed and can be justify through the observation described by Azzali [3] in *Vesperugo savi* and *Vesperugo piccolo.* He observed such cytological change in LH cell and suggest that the delayed ovulation in these bats may be due to insufficient quantities of LH hormones.

In *Taphozous longimanus* [21] LH cells in the pars distalis of female bat during early pregnancy are large and show inconspicuous Golgi apparatus. Mitochondria are round with lamellar cristae. The cisternae of rough surfaced endoplasmic reticulum are dilated and are distributed throughout the cytoplasm. Large numbers of secretory granules (250-350 nm) of varying electron density are observed throughout the cytoplasm. However, during mid-pregnancy, Golgi zone is well developed. The cytoplasm is vacuolated because of dilation of cisternae of the rough endoplasmic reticulum. Secretory granules are very few and are distributed throughout the cytoplasm. During late pregnancy, hypertrophied LH cells show rough endoplasmic reticulum occupying a large part of the cytoplasm, displacing other cell constituents. Cytoplasm is extremely vacuolated due to dilation of cisternae of rough endoplasmic reticulum. The hypertrophy of LH cells is associated with the filigreed cytoplasmic pattern giving a highly bizarre appearance to the cell as seen in the FSH cells during late pregnancy. The secretory granules are small and less in number and are distributed towards the periphery of the cell.

During anestrus period the LH cells are very few in number, while during estrus it increased in number and size.

In *Rousettus leschenaultia* [5] LH cells are angular in shape with secretory granules 100-150 nm in diameter. These secretory granules are of equal density and are irregularly distributed throughout the cytoplasm. Mitochondria are elongated. Golgi apparatus is prominent. In *S. heathi* [30,29] LH cells are seen with secretory granules 175-350 nm in diameter and are irregularly distributed throughout the cytoplasm. The mitochondria are elongated or round shaped and Golgi complex is conspicuous.

The ultrastructural characteristics exhibited by LH cells of different species of bats are similar to the ultrastructural features exhibited during present investigation in *Taphozous nudiventris kachhensis*, and supporting the present observations.

In the present study ultrastructural feature of LH cells indicates that cells are synthetically active during pregnancy and are hypertrophied at the end of pregnancy. LH cells may stimulate the luteal cell during pregnancy to secrete progesterone.

5. Conclusion

Ultrastructural features of FSH cells indicate that they are synthetically very active during estrus and pregnancy when ovary shows folliculogenesis and a Graafian follicle. Our observations suggest that the hypertrophied FSH cells at late pregnancy are necessary for maintaining Graafian follicle in the contra lateral ovary, which is going to ovulate. The LH cell in this species of bat shows well developed cell organelles such as Mitochondria, Golgi body, Rough endoplasmic reticulum, Smooth endoplasmic reticulum and these organelles indicates that these cells are very active.

References

[1] Selim A, El-Nahas E. Characterization of the Pars Distalis of the Egyptian Insectivorous Bats Taphozous Nudiventris by Using Both Ultrastructure and Histological Structure 2012;1:212, http://dx.doi.org/10.4172/scientificreports.212.

[2] Anthony ELP. Endocrinology of reproduction in bats: Central control. In: Crichton EG, Krutzsch PH, editors. In: Reproductive Biology of Bats. London, UK,: Academic Press,; 2000.

[3] Azzali G. Cytologia adenopofisaria dei chirotteri con prticolare reguardo alle cellule FSH, LH, ACTH, LTH. Ateno parmence Acta Bio-Med 1971;42:169–229.

[4] Badwaik NK. Cytology and seasonal changes of the pituitary of the emballonurid bat. Taphozous melanopogon (Temnick). Proc. Indian Acad. Sci. (Anim Sci) 1988;97:479–89.

[5] Bhiwgade DA, Akolkar VV, Menon SN, Mankar AP. Ultrastructural and functional characteristics of anterior pituitary cells in the Indian fruit bat, Rousettus leschenaulti (Desmarest). Acta Anat. (Basel) 1989;135:129–41.

[6] Farquhar MG, Rinehart JF. Electron microscopic studies of the anterior pituitary gland of castrate rats. Endocrinology 1954;54: 516–41.

[7] Herbert DC. Immunocytochemical evidence that luteinizing hormone (LH) and follicle stimulating hormone (FSH) are present in the same cell type in the rhesus monkey pituitary gland. Endocrinology 1976;98:1554–7.

[8] Herlant M. Electron microscope studies on the adenohypophysis of *Myotis myotis* during gestation. Gen. Comp. Endocr 1962;2:631.

[9] Herlant M. Apport de la microscopic electronique a l'etude du lobe anterior de l'hypophyse; in Cytoloie de l'adenohypophyse. C.N.R.S. Paris; 1963. p. 73–9.

[10] Herlant M. Cycle sexual chezies chiropters de regions temperees in cycle. In: Canivence R, editor. Genitaux saisonniers de Mammiferes. Paris: Masson et cie; 1968. p. 111–31.

[11] Herlant M. L'adenohypophyse de la chauve souris, Myia myetis au coura de la gestation. In: Titibach M, editor. In third European Regional Conference on Electron Microscopy, (. Prague: Academy of Science; 1964. p. 475–6.

[12] Ishibashi T, Shiino M. Sub cellular localization of prolactin in the anterior pituitary cells of the female Japanese house bat. Pipistrellus abramus. Endocrinology 1989;124:1056–63.

[13] Kmmrosumi K. Fumictiomial classificatiomi of tell types of the amiterior pitumitary gland accomplished by electron microscopy, 1 a*rch*. Hislol. Jap 1968;9:329.

[14] Kwiecinski GG, Damassa DA, Gustafson AW. Patterns of plasma sex hormone-bindingglobulin, thyroxine and thyroxine-binding globulin in relation to reproductive state and hibernation in female little brown bats. Journal of Endocrinology 1991;128:63–70.

[15] Martin L, Bernard RTF. Endocrine regulation of reproduction in bats: The role of circulating gonadal hormones. In: Crichton EG, Krutzsch PH, editors. In: Reproductive Biology of Bats. San Diego, USA: Academic Press; 2000. p. 27–64.

[16] Mikami S, Chiba S, Hojo H, Taniguchi K, Kubokawa K, Ishii S. Immunocytochemical studies on the pituitary pars distalis of the Japanese long-fingered bat, Miniopterus schreibersii fuliginosus. Cell Tissue Res 1988;251:291–9.

[17] Moriarty GC. Immuno cytochemistry of the pituitary glycoprotein hormones. J Histochem Cytochem 1976;24:846–63.

[18] Muniz E, Jiménez L, Gragera R, Fernandéz A, Rua C. Ultrastructural changes in the gonadotrophic andprolactin cells of *Myotis myotis* under experimental conditions. Funct. Dev. Morphol 1991;1:15–8.

[19] Murphy BD, James DA. Cells of the adenohypophysis of the mink (*Mustela vision*) identified by immune histochemical and functional criteria. Acta Anat 1976;94:184–203.

[20] Nakane PD. Classification of the anterior pituitary cell types with immunoenzyme and histochemistry. J Histochem Cytochem 1970;18:9–20.

[21] Nerkar AA. Electron microscopic studies on the endocrine gland and reproductive organs of Emballonurid female bat *Taphozous longimanus* (Hardwicke) during reproductive cycle. Ph.D. Thesis submitted to Rashtra sant tukdoji maharaj. Nagpur University. Nagpur; 2007.

[22] Nerkar AA, Gadegone MM. Ultrastructural characterization of the pars distalis of the Indian female sheath-tailed bat, Taphozous longimanus (Hardwicke). Int. J. Morphol 2010;28(3):787–801.

[23] O'Brien GM, McFarlane JR, Kearney PJ. Pituitary content of luteinizing hormone reveals species differences in the reproductive synchrony between males and females in Australian flaying- foxes (genus Pteropus). Reprod. Fertil. Dev 2003;15:255–61.

[24] O'Brien GM. Comparative morphology of the pituitary gland in Australian flying foxes (Megachiroptera: genus Pteropus). Anat. Rec 1996;244:70–7.

[25] Peyre A, Herlant M. Ovo-implantation différeé etcorrélations hypophyso-génitales chez la femelle du Minioptère (*Miniopterus schreibersii B*). C. R. Hebd. Seances Acad. Sci. D 1963;257:524–6.

[26] Purves HD. Morphology of the hypophysis related to its function. In: Young WC, editor. Sex and Internal Secretion. London: B. & Cox, T. Ltd; 1961. p. 162–229.

[27] Richardson, The anterior Pituitary and reproduction in Bats. J. Reprod. Fert 1979;56:379–89.

[28] Seraphim ER. Endocrine Interaction during different phases of the Female Reproductive Cycle in *Hipposideros lankadiva* (Kelaart), Ph. D. thesis,. Nagpur: RTM Nagpur University; 2004.

[29] Singh UP, Krishna A. Pituitary adreno corticotropic (ACTH) cells during reproductive cycle in a Vespertilionid bat, Scotophilus heathi. Acta Biol. Hung 1997;48:409–20.

[30] Singh UP, Krishna A. Identification, localization and distribution of pituitary cell types in female vespertilionid bat, Scotophilus heathi: A combined histochemical, immunocytochemical and electron microscopic study. Proc Indian Natl Sci Acad 1994;60:115–27.

[31] Sonwane DP. Endocrine Regulation of Reproduction in the Indian Female Vampire Bat *Megaderma lyra lyra* (Geoffroy). Ph.D. thesis submitted to Rashtra sant Tukdoji Maharaj,. Nagpur University, Nagpur, Maharashtra, India; 2010.

[32] Van Oordt PGWJ. Nomenclature of the hormone producing cells in the adenohypophysis. A report of the international committee for nomenclature of the adenohypophysis. Gen Comp Endocrinol 1965;5:131–4.