

Fine Structural Characteristics and the Regulatory Mechanisms of Adrenal-Cortical Region of Adult Male Fruit Bat, *Rousettus leschenaulti* (Desmarest)

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Abstract

The present paper describes the fine structural characteristics of the adrenal-cortical region of adult male fruit bat *Rousettus leschenaulti*. The functional significance of the secretions of the adrenal-cortical region in reproduction is discussed. This study was carried out in the Indian fruit bat *R. leschenaulti* (Desmarest) because of the unique aspects of reproduction of this animal. It shows two breeding cycles, one in the autumn and the other in the spring. The adrenal gland of *Rousettus* undergoes remarkable cyclical changes in relation to the reproductive cycle. The cortex and medulla are clearly demarked. During the inactive and pre-breeding period, the zona glomerulosa is made up of polyhedral cells arranged in the form of acini. On the other hand during the breeding period it is highly vascularized. It is composed of compactly arranged dark and light polyhedral cells. Smooth endoplasmic reticulum in the form of circular vesicles, is well developed during breeding phase. The zona fasciculata is a larger zone in the cortex. During inactive period, it is made up of polyhedral cells and during pre-breeding and breeding periods cells are polygonal in shape with spherical nucleus. The zona fasciculata is richly vascular during the breeding period. The cells of zona fasciculata during the breeding period have prominent smooth endoplasmic reticulum. Mitochondria, with tubular and vesicular cristae and a large number of lipid droplets, suggest increased rate of synthetic activity. The close association of smooth endoplasmic with mitochondria and lipid droplets indicate that these cells are actively involved in steroid biosynthesis. Steroidogenic organelles are well developed in the cells of reticularis during the inactive prebreeding and active breeding period. All these organelles suggest that cells of glomerulosa are active in synthesizing steroid hormones. The cisternae of smooth endoplasmic reticulum lie close to the nucleus, mitochondria and lipid droplets. This association most likely represents the conversion of steroids to lipid droplets for storage or the reverse trend indicates removal of sterol for steroidogenesis.

Keywords: Adrenal Cortex, Bat, Reproductive Cycle

1. Introduction

In spite of the worldwide distribution and diversity of order Chiroptera, relatively little attention has been given to understanding of reproduction in their males. Mammalian adrenal gland is unique among vertebrates in that the steroidogenic and chromaffin cells are clearly separated as cortex and medulla respectively¹. The

fine structure of the adrenal gland has been studied elaborately in a number of mammals such as: rats^{2,3}; dormouse⁴; goats⁵; horses⁶ and human^{7,8}. However, there is only scanty information available on the fine structure of the adrenal gland of bats, e.g. *Miniopterus schreibersii*⁹, *Vesperugo savi*, *V. piccolo*¹⁰, *Taphozous nudiventris*¹¹, *T. longimanus*¹² and *T. kachhensis*¹³. Morpho-histological studies on the adrenal gland of male and female bats,

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and ultrastructural characteristics of mammalian adrenal gland in general and those of bats in particular, suggest that zona fasciculata is the main engine of steroid hormone synthesis^{2,3,7} whereas zona glomerulosa is the site of cell proliferation, recruitment, differentiation and aldosterone biosynthesis and secretion⁴.

The relationship between reproductive function and high levels of glucocorticoids reported in bats represents an interesting model for further studies of the physiological role of the adrenal gland on reproductive timing and modulation in bats¹⁴. Therefore, an elaborate study of the structural and functional characteristics of the adrenal cortical region of the *Rousettus* during different phases of the reproductive cycle has been undertaken to find the probable role of secretory cells in the control of reproductive physiology of this species of bat.

2. Materials and Methods

The specimens of Indian fruit bat, *R. leschenaulti* (Desmarest), were collected from Nagpur (MS), India, throughout the year, representing different reproductive states. Adult males were trapped and brought alive in the laboratory with minimum stress. Uninterrupted supply of food and glucose water was provided. Five bats from each of sexually quiescent, recrudescing and sexually active males were sacrificed by cervical dislocation under chloroform anaesthesia. The adrenal glands were dissected out. For the electron-microscopic studies, the tissues were fixed in fresh ice-cold 3% glutaraldehyde for three hours and then four hours in 0.1 M cacodylate buffer. The tissues were washed in buffer and then post-fixed for one to two hours in 1% 0.067 M cacodylate-buffered osmium tetroxide. After dehydration with graded series of alcohol, the tissues were cleared in propylene oxide solution and embedded in Araldite resin which would be polymerized at 60°C. Then, ultrathin sections from selected blocks were cut with glass knife and picked up on 400-mesh copper grids. Sections were double stained with 10% alcoholic uranyl acetate for 20 min and in Reynold's lead citrate for 10 min. The sections were examined in a JEM Jeol-100s electron microscope (Japan) at 80KV accelerating voltage and photographed.

3. Results and Discussion

R. leschenaulti is a seasonally dioestrous tropical species (Bimodal breeding sequences), which breeds twice in a year. The bat has two breeding patterns one in the autumn

and the second one in the spring. Adrenal gland shows two distinct zones, the outer cortex region and inner medulla region. The medulla is completely surrounded by the cortex. The cortical region is further divided into three zones, zona glomerulosa, zona fasciculata and zona reticularis.

3.1 Zona Glomerulosa

3.1.1 Reproductively Inactive Period

During the inactive period, the zona glomerulosa is made up of acini, where each acinus is made up of 4-5 cells. The nucleus is spherical in shape and possesses prominent nucleolus and chromatin clumps in nucleoplasm. The smooth endoplasmic reticulum is in the form of vesicles which are distributed throughout the cytoplasm. Mitochondria are circular or spherical with vesicular cristae distributed throughout the cytoplasm and associated with numerous lipid droplets. Lysosomes are seen in the cytoplasm. The newly synthesized lipid droplets have low density and a light refractile surface while mature lipid droplets are relatively dense (Figure 1).

3.1.2 Prebreeding Period

During the prebreeding period, the cords of zona glomerulosa have a group of four to six cells arranged

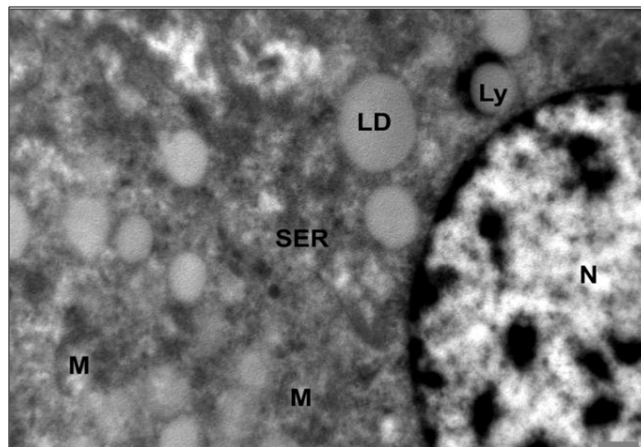


Figure 1. Electron micrograph of zona glomerulosa of adrenal cortex during the reproductively inactive phase. Note nucleus (N) with chromatin clumps at the periphery, many large Lipid Droplets (LD) associated with large number of round- to oval- shaped mitochondria (M) with tubular cristae whereas cisternae of tubular or vesicular Smooth Endoplasmic Reticulum (SER) are scattered throughout the cytoplasm. x15000.

in circular cords or acini-like structures. Each cell is bounded by a plasma membrane of uniform thickness and separated from adjacent cells by a space of uniform width. The nuclei of the cells of zona glomerulosa are round to oval in shape. The nucleus shows clumps of chromatin material at the periphery and the flakes of chromatin material are seen scattered through the nucleoplasm. Mitochondria are spherical to oval in shape with lamellar cristae distributed throughout the cytoplasm. Smooth endoplasmic reticulum is in the form of vesicles distributed throughout the cytoplasm. A large number of pleomorphic lipid droplets are present in the cytoplasm. Lysosomes in close contact with lipid droplets are also observed in the cytoplasm. There is an association among mitochondria, smooth endoplasmic reticulum and lipid droplets (Figure 2).

3.1.3 Active Breeding Period

During the active breeding period, the cords of zona glomerulosa have groups of three to four cells arranged in circular cords or acini-like structures. The nuclei are round to oval shape. The nucleus shows clumps of chromatin at the periphery and flakes of chromatin material are seen scattered through the nucleoplasm. The cells of zona glomerulosa have moderate to high amounts of tubular profiles of smooth endoplasmic reticulum scattered throughout the cytoplasm. The lipid droplets are few and usually appear as large vacuoles. Many lysosomes are observed in the cytoplasm (Figure 3).

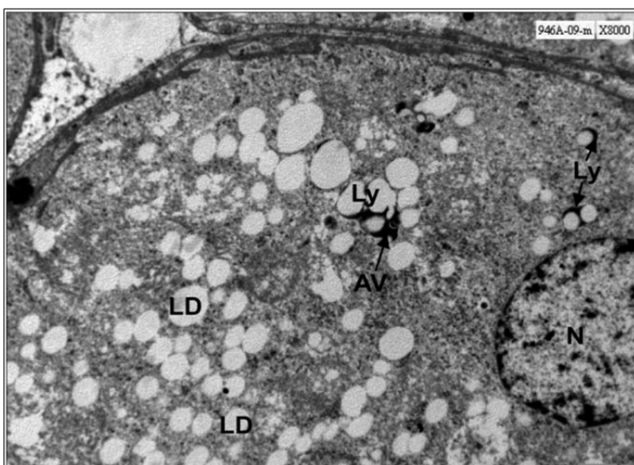


Figure 2. Electron micrograph of zona glomerulosa of adrenal cortex during the pre-breeding period showing nucleus (N). Note the tubular or vesicular cisternae of Smooth Endoplasmic Reticulum (SER) scattered throughout the cytoplasm. Many large Lipid Droplets (LD) and Lysosome (Ly) are also seen. x8000.

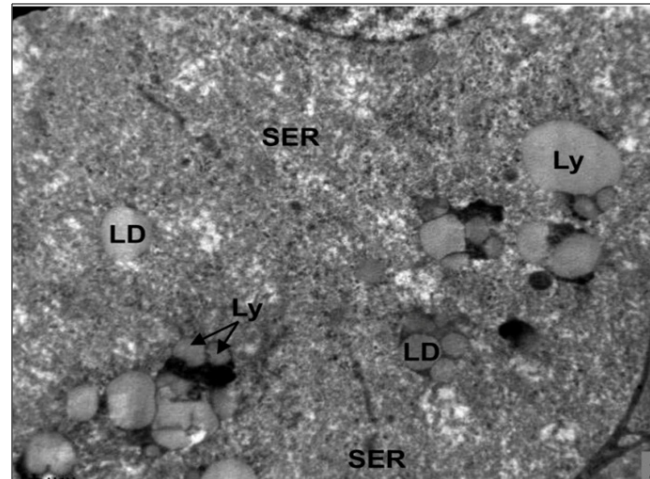


Figure 3. Electron micrograph of adrenal cortical region zona glomerulosa during the active breeding period. Note the presence of many tubular or vesicular cisternae of Smooth Endoplasmic Reticulum (SER) scattered throughout the cytoplasm. Numerous Lipid Droplets (LD) and Lysosomes (Ly) or vacuolated dense bodies are also seen. x10000.

3.2 Zona Fasciculata

The zona fasciculata is the largest zone in the cortex, and is made up of polyhedral cells. The cells are larger than those of zona glomerulosa.

3.2.1 Reproductively Inactive Period

During the inactive breeding period, the nucleus is large and spherical, and has a prominent nucleolus. The Golgi complex is inconspicuous. The smooth endoplasmic reticulum is in the form of small circular vesicles distributed throughout the cytoplasm. Numerous mitochondria are randomly distributed throughout the cytoplasm. They appear as profiles of various shapes, from circular to cylindrical. The cristae are tubular or vesicular. Numerous lysosomal bodies are present. Enormous lipid droplets are seen scattered throughout the cytoplasm (Figure 4).

3.2.2 Pre-Breeding Period

During the pre-breeding period, the polygonal cells of the zona fasciculata are large and vacuolated and arranged in groups or cords. The short cords merge among each other and are only one to two cells thick. The space between the cords is filled with blood. The nuclei are generally more regularly round than those in the zona glomerulosa. Chromatin clumps are observed at the periphery of the

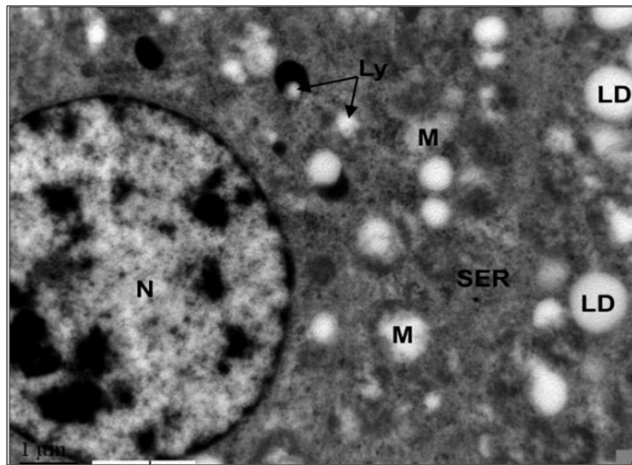


Figure 4. Electron micrograph of zona fasciculata adrenal cortex during the reproductively inactive period. Note the large nucleus (N) with chromatin clumps scattered throughout the nucleoplasm. Several spherical Lipid Droplets (LD) are present in close association with smooth endoplasmic reticulum (SER) and round to elongated mitochondria (M) with tubular cristae. A few Lysosomes (Ly) are also seen.

nucleus and are seen scattered in the nucleoplasm. Nucleolus is well developed. Mitochondria are randomly distributed in the cytoplasm. Their sections appear in various profiles, circular to elongate. The cells of zona fasciculata have tubular profiles of smooth endoplasmic reticulum scattered in the cytoplasm. Lipid droplets are abundant in this zone and they are larger than those in the zona glomerulosa. Lysosomal bodies are also noticed. The lipid droplets are observed in close association with the smooth endoplasmic reticulum and mitochondria (Figure 5).

3.2.3 Active Breeding Period

During the active breeding period, the cells of the zona fasciculata make up most of the thickness of the cortex. The most striking feature of the cytology of this zone is the elaborate smooth endoplasmic reticulum and abundant lipid droplets. Mitochondria of the zona fasciculata are large and more pleomorphic than those of the glomerulosa. The mitochondria have tubular cristae. In some mitochondria the cristae are collapsed. The smooth endoplasmic reticulum is made up of tubular or vesicular elements. Many ribosomes are free in the granular cytoplasm. Lipid droplets are more abundant than in the cells of zona glomerulosa. These are observed

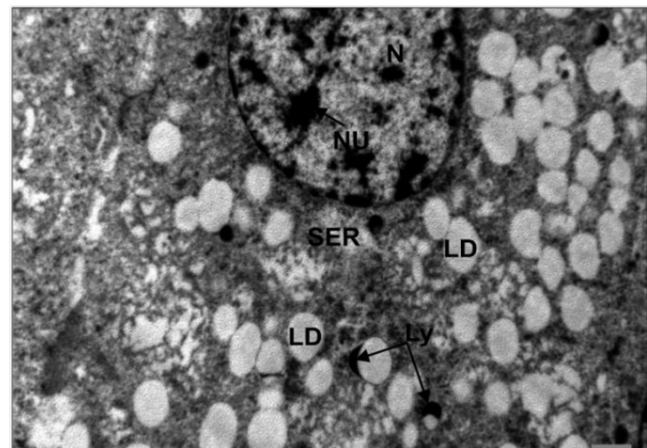


Figure 5. Electron micrograph of zona fasciculata of the adrenal cortex during pre-breeding period showing nucleus (N) with Prominent Nucleolus (NU) and chromatin clumps. Note the large, spherical Lipid Droplets (LD), many lysosomes and Smooth Endoplasmic Reticulum (SER) scattered throughout the cytoplasm. x10000

in close associations with smooth endoplasmic reticulum and mitochondria (Figure 6).

3.3 Zona Reticularis

The zona reticularis is the innermost zone. The cells are smaller than in the other zones.

3.3.1 Reproductively Inactive Period

During the reproductively inactive period, the nucleus is spherical and the nucleolus is distinct. The smooth endoplasmic reticulum is in the form of small circular vesicles distributed throughout the cytoplasm. The mitochondria are numerous and small in size with vesicular cristae and are associated with lipid droplets. These droplets are mostly spherical to ovoid in shape with an irregular boundary, and contain material of variable electron density. Secondary lysosomal bodies are numerous. A large blood vascular space containing blood is observed in the zona reticularis and medulla (Figure 7).

3.3.2 Pre-Breeding Period

During the pre-breeding period, the cells of zona reticularis are arranged as radial cords, one or two cells thick and lie between vascular channels which converge towards the medulla. The nuclei are round to oval in shape with prominent nucleolus. A thin layer of chromatin material is observed at the periphery of the

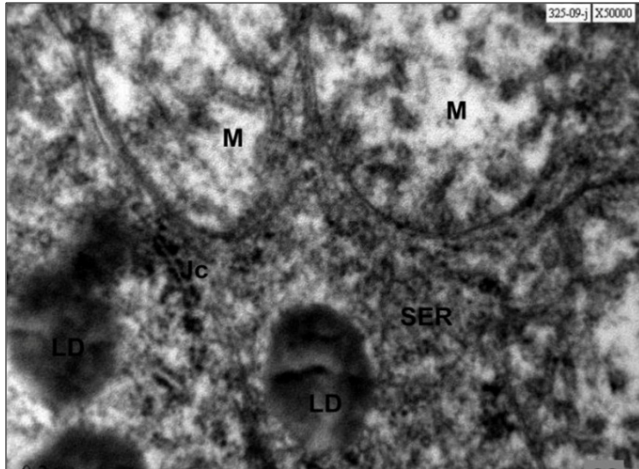


Figure 6. Electron micrograph of zona fasciculata of adrenal cortex during the active breeding period. Note the large vacuolated mitochondria (M) with collapsed cristae and Lipid Droplets (LD) present in the cytoplasm. The Smooth Endoplasmic Reticulum (SER) is well developed. It is in the form of tubules scattered throughout the cytoplasm. x50000.

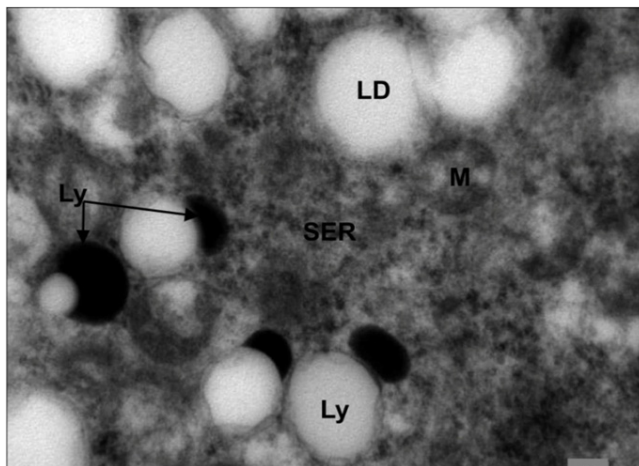


Figure 7. Electron micrograph of zona reticularis adrenal cortex during reproductively inactive period. Note the numerous secondary Lysosomal bodies (Ly), Round Lipid Droplets (LD) which are scattered throughout the cytoplasm associated with the spherical mitochondria (M) possessing tubular cristae. Note the tubular network of Smooth Endoplasmic Reticulum (SER). X25000.

nucleus. Mitochondria are small with vesicular cristae and the smooth endoplasmic reticulum is in the form of vesicles distributed throughout the cytoplasm. Rough

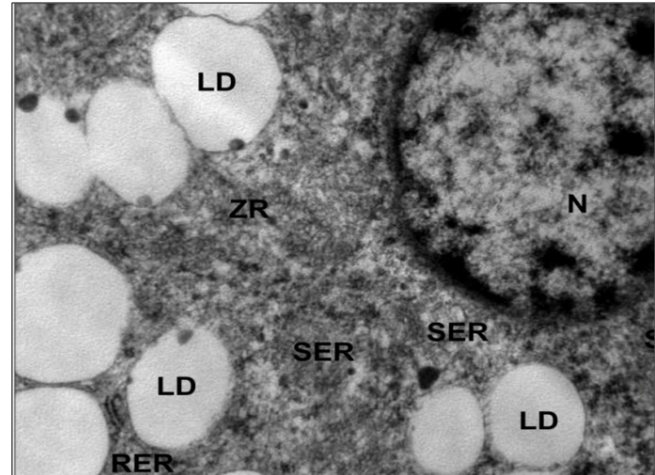


Figure 8. Electron micrograph of zona reticularis of adrenal cortex during the pre-breeding period. Note large nucleus (N), several spherical Lipid Droplets (LD) that occur in close association with the Smooth Endoplasmic Reticulum (SER) scattered throughout the cytoplasm. x25000.

endoplasmic reticulum is in the form of short cisternae dotted with ribosomes. A large number of lipid droplets are found in the cells. These droplets are pleomorphic with irregular boundaries surrounded by smooth endoplasmic reticulum.

The most striking feature of the cytology of this zone is the elaborate smooth surface endoplasmic reticulum and abundant lipid droplets and mitochondria (Figure 8).

3.3.3 Active Breeding Period

During the active breeding period, the cells of zona reticularis contain oval or round nuclei with a rim of heterochromatin just underlying the nuclear membrane. Heterochromatin clumps are distributed in the nucleoplasm. Steroidogenic organelles are well developed in the cells of reticularis. Mitochondria are spherical or elongated, distributed throughout the cytoplasm. They have vesicular cristae. Profiles of smooth endoplasmic reticulum are in the form of vesicles or tubules distributed throughout the cytoplasmic matrix. Rough endoplasmic reticulum is well developed and consists of short to elongated cisternae dotted with ribosomes. A large number of pleomorphic lipid droplets are present in the cytoplasm. Lysosomal bodies are observed in cytoplasm (Figure 9).

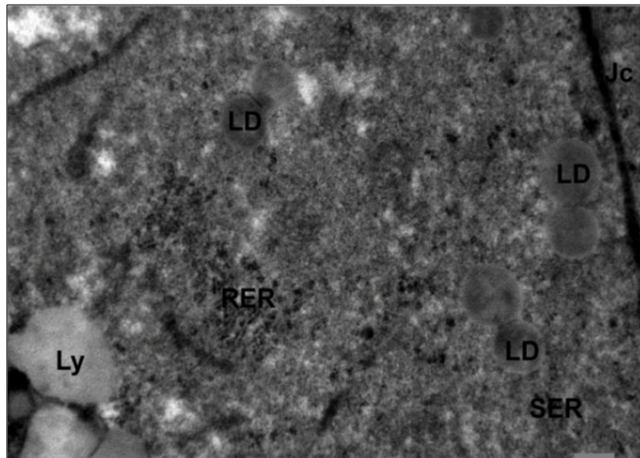


Figure 9. Electron micrograph of zona reticularis of the adrenal cortex during the active breeding period. Note the large number of pleomorphic Lipid Droplets (LD), cisternae of Smooth Endoplasmic Reticulum (SER) and 2 to 3 lysosomal bodies (Ly). x20000.

4. Discussion

The cortical cells are equipped with all necessary organelles associated with steroidogenesis. Thus, it is suggested that the adrenal cortex of *R. leschenaulti* is not only responsible for production of glucocorticoids but also for the production of sex steroid hormones, which are required to support continuous process of reproduction in this species of bats. There is a close association of mitochondria, smooth surfaced endoplasmic reticulum and lipid droplets in the adrenocortical cells during the pre-breeding period and the increased complement of these organelles during the active breeding period indicates heightened steroid biosynthesis. The ultrastructural characteristics of adrenal cortical cells suggest that these cells are engaged in steroid and protein synthesis.

5. References

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