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**HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES
ON ADRENAL CORTEX OF EGYPTIAN BALADI GOATS**

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The aim of this study was to investigate the structure of the goat adrenal cortex. For this purpose, thirty six adrenals were collected from adult goats. The tissues were processed for both light and electron microscopy. The adrenal cortex is composed of three distinct zones. The outer zone is the zona glomerulosa and its cells arranged in glomerules. The zona fasciculata is the thickest zone and is composed of columns of secretory cells separated by prominent capillaries. The cells are polygonal and have many intracellular lipid droplets. The zona reticularis is also composed of polygonal cells, whose arrangement is less linear and more as round nests or clumps of cells.

Ultrastructurally: zona glomerulosa are characterized by prominent mitochondria with tubular cristae, zona fasciculata have many cytoplasmic lipid droplets, mitochondria with vesicular cristae, and abundant smooth endoplasmic reticulum. The zona reticularis is distinguished by the presence of prominent lysosomes in the cytoplasm.

Baladi goats are the most versatile domestic animals in the Nile Valley of Egypt [1]. They are distributed across the country, especially dense in the Nile valley and delta and with lower concentration in the north-western coastal region and at oases [2].

Histological studies on the adrenal gland of goat are of critical importance for a plethora of many biological processes such as stress response, immune function, cardiovascular regulation and metabolism [3]. The adrenal cortex is required for life, particularly the secretion of aldosterone, but the functions of the medulla are not essential for life [4]. Hence, the adrenal glands are extremely labile organs that stereotypically react to environmental changes.

The adrenal cortex consists of three histologically distinct zones: the zona glomerulosa (ZG), which is located immediately below the capsule; the zona fasciculata (ZF), which is in the middle; and the zona reticularis (ZR), which is the innermost zone next to the medulla [5]. The aim of the present study is to clarify morphological and ultrastructural findings of the three zones of normal adrenal cortex from Egyptian Baladi goats. This would be a basis for comparison with other adrenal cortex investigations.

Material and methods

Thirty adrenal glands of Baladi healthy goats aged between 3 and 7 years were used in this study. They were collected from Zagazig abattoir. The adrenals were taken as soon as possible after slaughter of the animals. Twenty four adrenal glands were used for histological and histochemical studies. Six adrenal glands used for Ultrastructural studies.

For the histological investigation, the freshly collected specimens were fixed immediately in one or more of the following fixatives: 10 % buffered neutral formalin, Bouin's fluid, Helly's fluid and Susa fixative. Then the specimens were processed using the usual histological techniques till be embedded in paraffin wax, sectioned at 5-7 micrometer in thickness. The methods of processing and staining were quoted from [6] and [7].

The obtained sections were stained with:

1. Harris's haematoxylin and Eosin.
2. Van Gieson's stain.
3. Toluidine blue stain.
4. Periodic acid Schiff (PAS) technique.

For Transmission Electron Microscopic studies, Small pieces of adrenal tissues were collected immediately after slaughter. These specimens were fixed in 2% glutaraldehyde and processed for the ultrastructural study where the glutaraldehyde was diluted in sod. cacodylate buffer at PH 7.3 for 3 hours at room temperature, then washed in phosphate buffer, postfixed in 1% osmium tetroxide in 0.04 M cacodylate buffer at PH 7.3 embedded in Epon-araldite, then ultrathin sectioned mounted on copper grids and stained with uranyl acetate then with lead citrate, and examined by TEM Joel 100, the methods were quoted from (8).

Results

The adrenal gland is enclosed in clearly visible two-layered capsule, an outer fibrous and an inner cellular layers. In the fibrous layer the rough bundles of collagen fibrils and smooth muscle cells predominated over elastic fibres. The cellular layer consisted of loose connective tissue and large numbers of small and round cells, resembling undifferentiated cortical cells (Figure 1). The average thickness of the capsule was $134.39 \pm 0.35 \mu$ at 3 years age. In the surrounding connective tissue, a supracapsular network of blood vessels and nerve fibres was detectable (Figure 2). Externally the adrenal gland was enclosed by capsula adiposa (Figure 2).

Originating from the connective tissue capsule, narrow and broad trabeculae spread towards the cortex (Figure 3). The trabeculae were especially thick at the beginning of their course, later they became somewhat thinner and reached the border between the cortex and the medulla as thin septa.

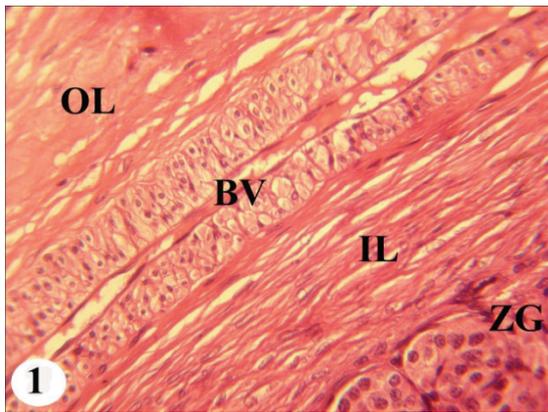


Figure 1 - A photomicrograph for a section in the adrenal gland of a goat aged three years, showing two-layered capsule of the adrenal gland, an outer fibrous layer (OL) and an inner cellular layer (IL). Zona glomerulosa (ZG), blood vessel (BV). Stain: H&E. Obj. x 100: Oc. x 10.

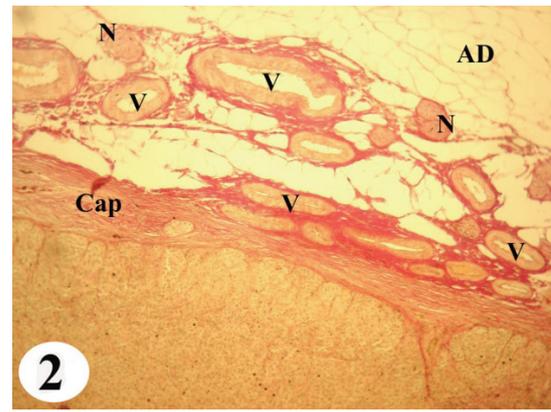


Figure 2 - A photomicrograph for a section in the adrenal gland of a goat aged three years, showing adrenal gland capsule (Cap) with numerous blood vessels (V), nerve fibres (N). Capsula Adipose (AD). Stain: Van Geison stain. Obj. x 10: Oc. x 10.

Clusters of cells, which by their appearance and staining properties resembled the cortical cells, and clusters of cells which looked like medullar cells could be found within the capsule (Figure 4).

The adrenal gland had three cortical zones clearly identifiable by arrangement and stainability of cells: zona glomerulosa, zona fasciculata and zona reticularis. The widest one was zona fasciculata $912.331 \pm 1.63 \mu$, followed by zona reticularis $682.84 \pm 14.44 \mu$ and the narrowest one was glomerulosa $385.028 \pm 7.6 \mu$ (Figure 4).



Figure 3 - A photomicrograph for a section in the adrenal gland of a goat aged three years, showing Connective tissue capsule (Cap) on the gland surface from which a thick trabeculae (T) is extended toward the cortex. Zona glomerulosa (ZG), zona fasciculata (ZF). Stain: PAS technique. Obj. x 10: Oc. x 10.

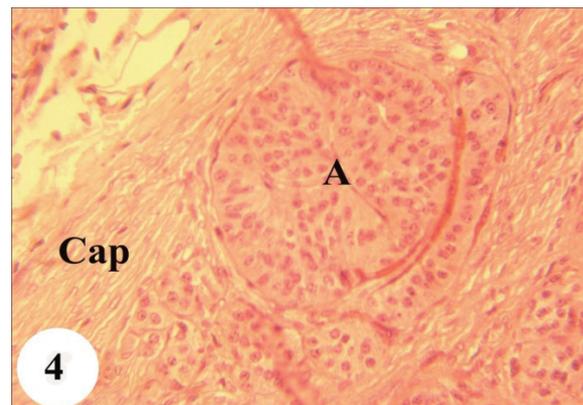


Figure 4 - A photomicrograph for a section in the adrenal gland of a goat aged three years, showing cortical cells aggregation (A) within the connective tissue capsule (Cap). Stain: H&E. Obj. x 40: Oc. x 10.

Subcapsularly lying the zona glomerulosa was created by oval or cuboidal epithelial cells collected in cluster (glomerules) surrounded by delicate connective tissue fibres (Figure 5). Blood capillaries could be found within the glomerules and between the neighbouring glomerules. The cells of zona glomerulosa had an acidophilic cytoplasm and oval nuclei (Figure 5). A small nucleolus could be seen within the nuclei.

The zona fasciculata was built of radially orientated cords of cells. It seemed that there were one or two cell rows in each cord (Figure 6). The cells were large, cuboidal or polygonal shaped with the centrally located light nuclei. Very often binucleate cells could be seen. The cells had many small lipid droplets (Figure 7).

The zona reticularis presented the deepest layer of the adrenal cortex. It consisted of interconnecting, irregular cords of small, polygonal cells with dark nuclei (Fig.8). Between the cords of cells the capillaries often appeared dilated, contrary to the capillaries of the zona glomerulosa and the zona fasciculata also there's further increase in the number of blood sinusoids was noted in the zona reticularis, making it the most vascularized of the three zones

Ultrastructural findings: The cells of zona glomerulosa characterized by large oval nuclei, a large number of mitochondria with tubulo-vesicular cristae and some lipid droplets. The profile of smooth endoplasmic reticulum presented either as small vesicles or flattened short sacs (Figure 9).

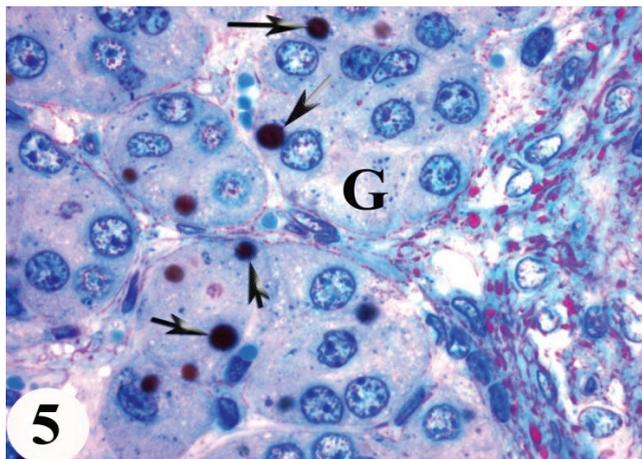


Figure 5 - A photomicrograph for a semithin section in the adrenal gland of a goat three years, showing a part of zona glomerulosa. The cells are large cuboidal with round or oval nuclei. Cells arranged in glomerules (G), surrounded by delicate connective tissue fibers. Lipid droplets (arrows). Stain: Toluidine blue X 1000.

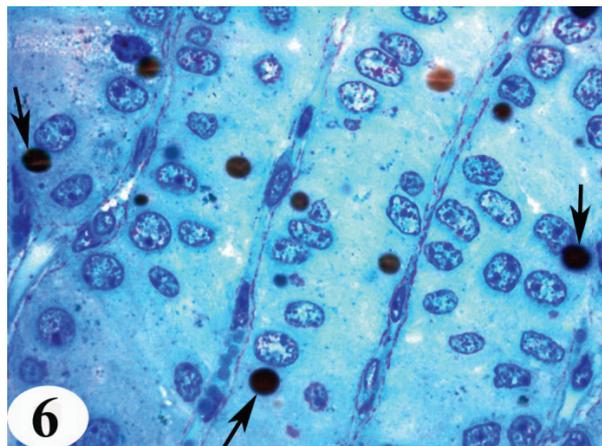


Figure 6 - A photomicrograph for a semithin section in the adrenal gland of a goat aged seven years, showing a part of zona fasciculata. The cells are radially arranged between blood capillaries separating them. Notice lipid droplets (arrows). Stain: Toluidine blue X 1000.

The zona fasciculata showed the characteristic ultrastructure, consisting of mitochondria with vesicular cristae and smooth endoplasmic reticulum in the form of a network of anastomosing branching tubules and numerous lipid droplets (Figure 10). The Golgi complex was frequently a prominent structure (Figure 11).

The cells of zona reticularis were smaller, their nuclei contained more condensed chromatin and the nucleolus was less prominent (Figure 12). In the cytoplasm, only a few lipid droplets were present, but abundant profiles of SER, mitochondria with tubulo-vesicular cristae could be seen and numerous lysosomes and lipofuscin granules (Figure 13).

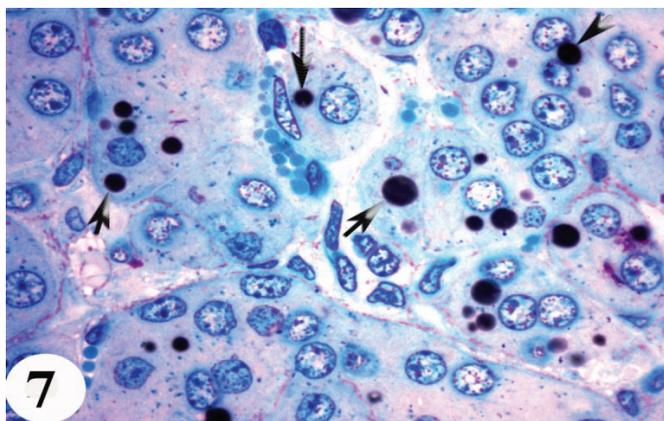


Figure 7 - A photomicrograph for a semithin section in the adrenal gland of a goat three years, showing a part of zona fasciculata. The cells are polygonal in shape. Their nuclei are rounded vesicular with prominent nucleoli. Notice the numerous lipid droplets (arrows) in the cytoplasm of most cells. Stain: Toluidine blue X 1000.

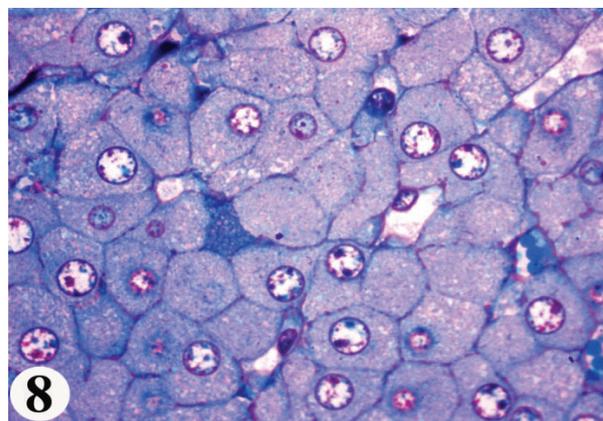


Figure 8 - A photomicrograph for a semithin section in the adrenal gland of a goat aged seven years, showing a part of zona reticularis. The cells are small polygonal with small dark nuclei giving a net-like appearance. Stain: Toluidine blue X 1000

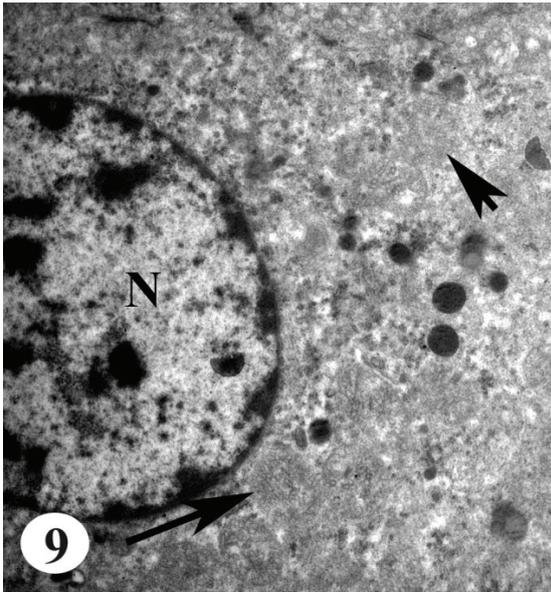


Figure 9 - An electron micrograph of glomerulosa cells of goat, showing mitochondria with tubular cristae (arrows) and vesicles of smooth endoplasmic reticulum are distributed throughout the cytoplasm. Nucleus (N). X 15000.

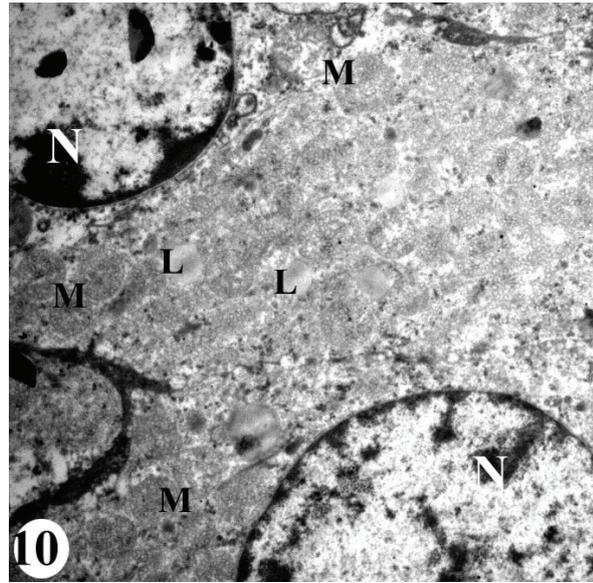


Figure 10 - An electron micrograph of fasciculata cells of goat, showing cytoplasm is packed with round Mitochondria (M) display vesicular cristae. Moderately electron lucent rounded lipid droplets (L) and nucleus (N). X 10000.

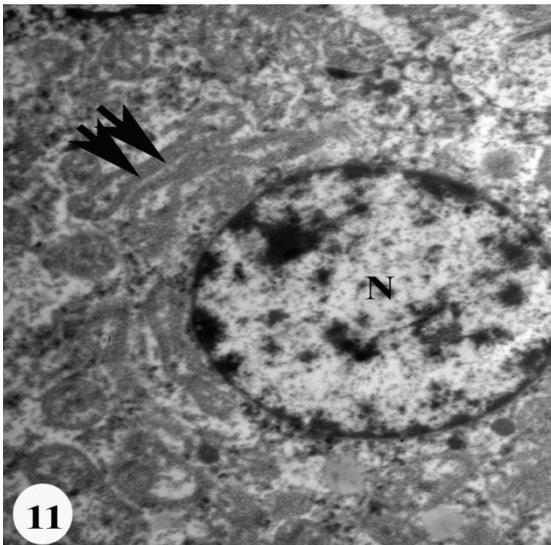


Figure 11 - An electron micrograph of fasciculata cells of goat, showing well developed Golgi complex (arrow). Nucleus (N). X 10000.

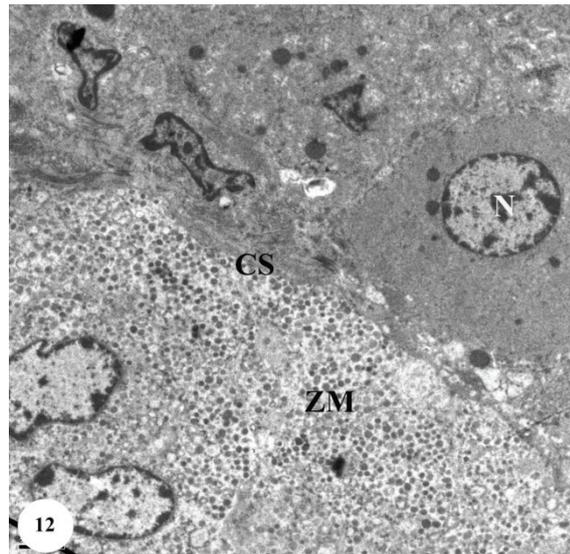


Figure 12 - An electron micrograph of reticularis cells of goat, showing small nuclei contained condensed chromatin with less prominent nucleolus (N). Lipid droplets. Connective tissue stroma between zona corticalis and zona medullaris (CS), zona medullaris (ZM). X 10000.

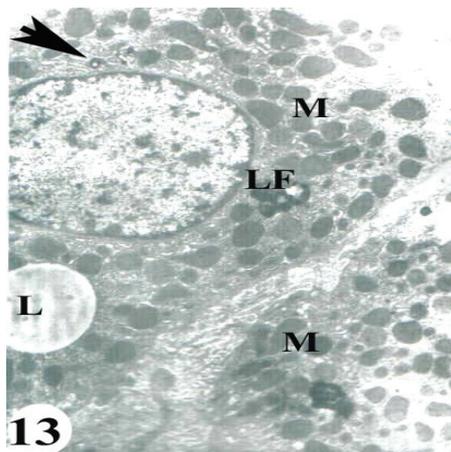


Figure 13 - An electron micrograph of reticularis cells of goat, showing several lipofuscin-pigment (LF) granules. Irregularly shaped mitochondria (M), lipid droplet (L), smooth endoplasmic reticulum (arrow), nucleus (N). X 10000.

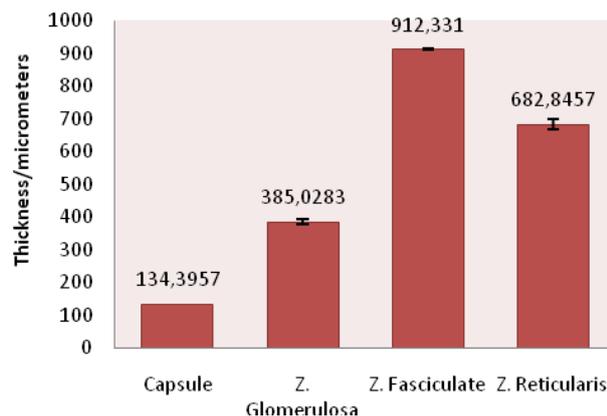


Figure 14 - Histogram showing the average values of thickness of capsule cortical zones in three years old goat adrenal gland.

Discussion

The high values of thickness of capsule and presence of cortical cells within the capsule indicate that the adrenal capsule is very active structure at this period. These results confirm the pivotal role of the capsule as pool of cells potent to proliferate, migrate centripetally and differentiate into cortical cells [9].

Our study has revealed that the goat adrenal cortex, as in other mammals, has three morphologically defined zones. These zones are an outer zona glomerulosa (ZG), a middle zona fasciculata (ZF), and an inner zona reticularis (ZR). This result also provided by (10) in Porcupine. In this study the area of cortex occupied by the glomerulosa, fasciculata and reticularis zones was $385.028 \pm 7.6 \mu$, $912.331 \pm 1.63 \mu$ and $682.84 \pm 14.44 \mu$ respectively.

Like in other mammalian species, in the goat adrenal cortex the best developed is the fasciculata zone, the cells of which are the largest and the most numerous (46%) among the parenchymal cells of the cortex. The zona reticularis cells comprise about 34.4% of all parenchymal cells of the cortex while glomerulosa cells only 19.4%. Thus, the zonal and cellular composition of the goat adrenal cortex resembles that of some other mammalian species [11].

Histological examination revealed that zona glomerulosa cells are cuboidal with oval nuclei and contain small amounts of lipids, as compared to those in the other cortical zones [12] in rat.

According to our research, cells in the zona fasciculata were polygonal with large, light nuclei and frequently had two nuclei.

The foamy appearance of the cells, described for domestic animals [13] and humans [14 and 15] caused by the presence of numerous empty vesicles from the dissolution of lipid droplets during routine tissue processing, were observed.

In this study ultrastructural features of the cortical cells can be very useful for their identification. All 3 zones have prominent mitochondria that can be distinguished by the shape of their cristae. Cells of the zona glomerulosa are characterized by prominent mitochondria with tubulo-vesicular cristae, whereas cells of the zona fasciculata have many mitochondria with vesicular cristae. The zona reticularis is distinguished by mitochondria with tubulo-vesicular cristae. These results are in accordance with [16]. It is believed that mitochondria play a major role in steroidogenesis, and the finding of specialized mitochondria in different zones of the adrenal cortex supports this concept.

The present study found that the cells of zona reticularis contain numerous lysosomes and lipofuscin granules with greater increase in aged animals. This is in agreement with [17]. The cell migration theory of the adrenal cortex (18) stated that cell proliferation occurs in the zona glomerulosa, or the intermediate zone between zona fasciculata and zona glomerulosa, and, as the cells differentiate, they move centripetally and die at zona reticularis, where they are removed by the macrophage. Whether for the purpose of clearance or as an additional modulation effect, adrenal macrophages attain their highest numerical density in zona reticularis.

Lipofuscin granules, a common structure in zona reticularis. They exhibit cellular remnants in a manner similar to those observed in hepatocytes, Leydig cells, cardiac muscle cells, neurons and corpus luteum cells, among others, which supports their origin in autolysosomes [17]. Our ultrastructural and Histological findings are consistent with this view.

In conclusion, despite a general resemblance in the structure of adrenal cortex, our results represent the first study on the adrenal cortex of Egyptian Baladi goats.

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Резюме

Целью данного исследования было изучение структуры коры надпочечников коз. Для этого было взято тридцать шесть надпочечников взрослых коз. Ткани были проанализированы под световым и электронным микроскопом. Кора надпочечников состоит из трех отдельных зон. Внешняя зона является клубочковой, ее клетки собраны в клубочки. Пучковая зона является самой плотной зоной и состоит из столбцов секреторных клеток, разделенных четко видимыми капиллярами. Клетки многоугольные. Имеются внутриклеточные капельки липидов. Сетчатая зона также состоит из многоугольных клеток, расположение которых менее линейно и более походит на круглые гнезда или скопления клеток.

Ультраструктурный анализ: клубочковая зона характеризуется четко различимыми митохондриями с трубчатыми кристами, в цитоплазме пучковой зоны имеется значительное количество липидных капель и гладкого эндоплазматического ретикулума. Сетчатую зону отличает наличие в цитоплазме четко различимых лизосом.

Тұжырым

Бұл жұмыста ешкілердің бүйрек үсті безі қабатының құрылымы зерттелген. Ол үшін отыз алты ересек ешкілердің бүйрек үсті бездері жиналып, олардың ұлпалары жарық және электрондық микроскоппен зерттеу үшін өңдеуден өткізілді. Бүйрек үсті безінің қабаты жеке үш бөліктен тұратындығы анықталды. Сыртқы қабат клеткалары шумақталып орналасқан ұлпаны құрайды. Ортаңғы қабаты будаланған клеткалар топтамаларынан тұрады және бұл қабатта секрет клеткаларының таяқшалары орналасқан. Сондай-ақ бұл қабатта липид тамшыларынан құралған полигональды клеткалар топтамалары болады. Ішкі қабаты да полигональды клеткалардан тұрады. Олар дөңгеленіп, ұяшық тәрізді орналасқан және ол торшалы қабат деп аталады.

Ультрақұрылымы: будаланған қабат митохондриялардың, көптеген цитоплазмалық липидті тамшылардың және эндоплазмалық ретикулум құрылымдарының көп болуымен сипатталады. Ішкі торшалы қабат цитоплазмасы лизосомды клеткалардың болуымен ерекшеленеді.