

Ultrastructural characteristics of the anterior pituitary gland of cattle in the definitive period of postnatal ontogenesis.

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Abstract. Today much attention is paid to the study of the pituitary ultrastructure of laboratory animals and humans. But there is not enough available literature to study the most important organ of internal secretion of productive animal. The aim of our work is to study the structure of the ultrastructure of cells of the anterior pituitary gland of cattle in the definitive period of postnatal ontogenesis. Histological, morphometric, and electron microscopic techniques were used to study the cellular composition of the anterior pituitary gland of cattle. It was revealed that the anterior lobe of the pituitary gland occupies 64 % of the entire pituitary parenchyma, while the posterior and middle lobes occupy 27% and 9%, respectively. After using general histological methods there are detected the functionally inactive chromophobes (41%) and chromophilic cells, which include acidophils (38%) and basophils (21%) in the anterior lobe of the pituitary gland. The electron microscopic studies in the parenchyma of the anterior pituitary gland let find Somatotropes that differ in the presence of a large number of secretory granules in the cytoplasm. Lactotropes are less common than somatotropes and differ from them in larger secretory granules. Corticotropes, gonadotropes and thyrotropes with a minimum content of secretory granules are the least detected. All identified endocrine cells are at different stages of functional activity.

1 Introduction

The pituitary gland is the main gland of internal secretion. It is involved in providing control of many vital processes, such as growth, development, homeostasis, reproduction and adaptation of the body to changing conditions of the external and internal environment [1-4]. It is well known that the pituitary gland in ontogenesis develops from the epithelial and neural rudiments, forming a complex morphological structure an organ of internal secretion. The main element of the adaptive-compensatory mechanism of the endocrine system of the body is the adenohipophysis [5]. The adenohipophysis consists of acinuses, which contain specialized cell types, each of which has its own unique hormonal function and characteristics [6]. The analysis of foreign and domestic literature showed insufficient information concerning the cell structure, differentiation, classification

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and structural features of the cell composition of the anterior pituitary gland in mammals, particularly in artiodactyls [7-15].

The aim of this work is to study the structure of cells of the anterior pituitary gland of cattle at a definitive age.

2 Material and methodology

The research material was the pituitary gland of 2-3 years aged cattle. The gland was extracted by dissection and it was weighed. For histological studies the material was fixed in Buena liquid, 10% of it in neutral formalin. After that material was carried out into paraffin. The research sections were prepared on a rotary microtome with a thickness of 5-6 microns; they were stained by hematoxylin and eosin using the Heidenhain's AZAN stain method.

Using transmission electron microscopy there was cut out pieces of the pituitary gland with a size of about 1 mm³, fixed with a 2.5% solution of glutaraldehyde on a phosphate buffer (PBS) for 1.5 hours at a temperature of 20°C-22°C. for investigation. After washing in PBS, 1% solution of osmic acid in PBS was added for 2 hours. For dehydration, biological objects were carried out in alcohols of increasing concentration and absolute acetone. After that, the objects were placed in molds, poured into epon at a temperature of 37° C for 24 hours, then for 48 hours at a temperature of 60 °C. The sections were made on a Leika EM UC6 ultramicrotome (Leica, Germany), which were additionally contrasted in lead citrate. The sections were studied and photographed using a transmission electron microscope Tecnai Spirit G2 (Philips, the Netherlands). The microscope includes a high-resolution digital camera SIS MegaView III 120 kV. The study of biological objects and the production of ultra-thin sections using transmission electron microscopy were carried out on the basis of the CCP "Modern Microscopy" of the Academy of Biology and Biotechnology of the Southern Federal University. Cell counting and karyometry were performed using an ocular camera UCMOS 03100KPA with licensed software "Altami Studio". Statistical processing of the obtained data was performed using the Microsoft EXCEL software package.

3 Research results

The pituitary gland is located in the pituitary fossa of the Turkish saddle of the sphenoid bone, surrounded laterally by a dense network of blood vessels and covered with a connective tissue diaphragm (Fig. 1, a). The average weight of the pituitary gland is 2.65±0.15 g.

The sagittal section clearly shows the boundaries of the pituitary lobes (Fig. 1, b), where the anterior pituitary lobe (1) occupies the majority (64%), while the posterior lobe (2) occupies the dorsocaudal location (27%). The middle lobe (3) is adjacent to the anterior lobe (9%), forming the pituitary fissure (5). The pituitary pedicle (4) has a dorsocranial position and connects to the gray hillock through an opening in the diaphragm. Dorsally, the pituitary gland is covered by a capsule of dense connective tissue (6).

The parenchyma of the anterior pituitary gland is represented by various cell populations. Heidenhain's AZAN stain method can revealed the layers of connective tissue (Fig.1 c, d), between which the adenocytes forms strands and clusters. Traditionally, the cells of the anterior pituitary gland using histological techniques are classified according to the ability of the cytoplasm to perceive a particular dye. Thus, the adenocytes are divided into: chromophobic, the cytoplasm of which remains free of dye.

Some authors believe that these cambial cells are necessary for the renewal of secretory cells of the pituitary gland. There is also an opinion that these cells have released their secret into the bloodstream after which the cytoplasm seems empty and chromophilic cell the cytoplasm of which perceives dyes. This type of cell is divided into acidophilic cells (which perceive acidic dyes) and basophilic cells (the cytoplasm is colored with basic dyes).

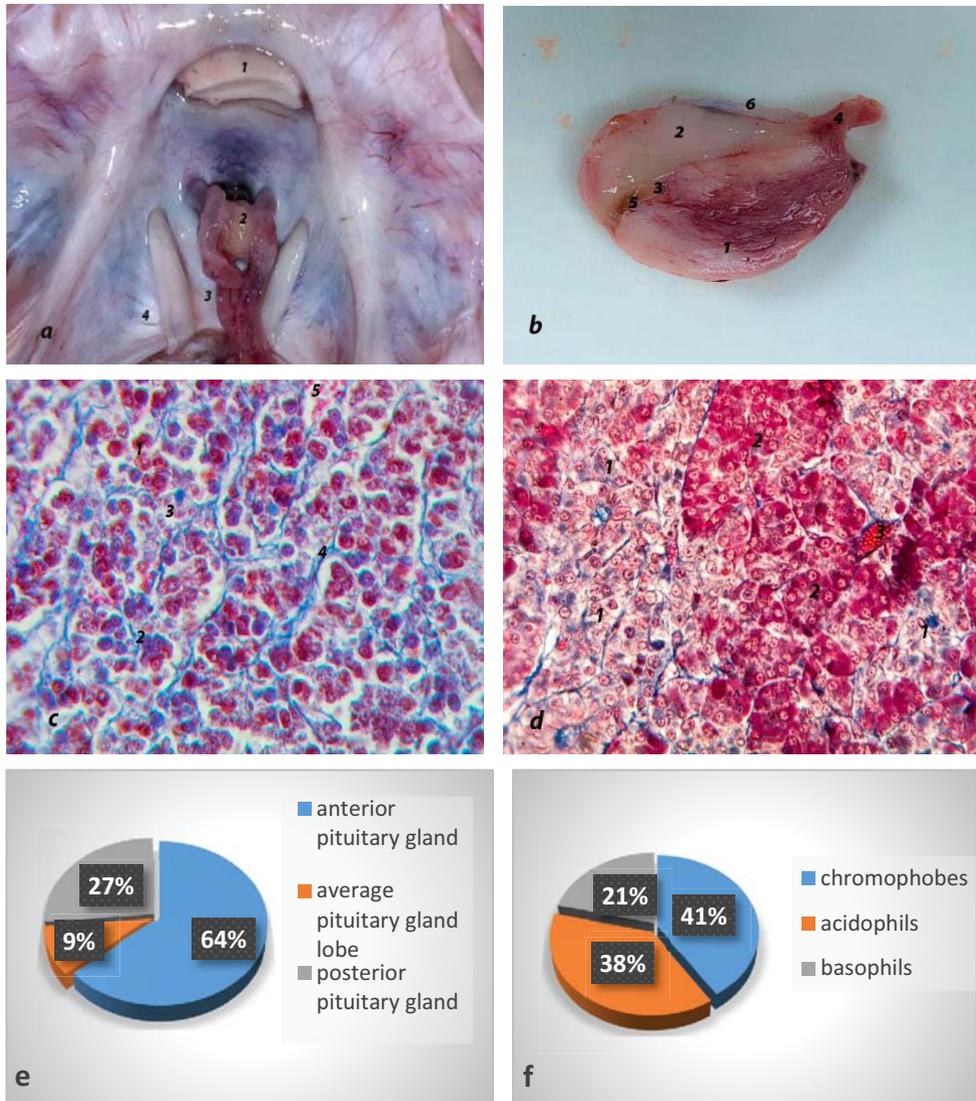


Fig. 1. *a* - the location of the pituitary gland in the skull. 1-the visual intersection, 2-the pituitary pedicle, 3-the location of the pituitary gland, 4 - the dura mater; *b* - Sagittal section of the pituitary gland of 2 years cattle. 1 - anterior pituitary lobe, 2-posterior pituitary lobe, 3-middle lobe, 4-pituitary pedicle, 5-pituitary capsule; *c* - Pituitary gland of 2 years cattle 1-acidophils, 2-basophils, 3-chromophobes, 4-connective tissue, 5-blood vessel. Fixation neutral formalin, Heidenhain's AZOCARMIN stain, uv. x400; *d* - Pituitary gland of 2 years cattle. 1-basophils, 2-acidophils, 3-blood vessel. Buen fixation, Heydenhein azocarmine staining, uv. x400; *e* - Percentage of the area of the pituitary gland of cattle; *f*-Percentage of the cells of the anterior pituitary gland of cattle.

Among the cells of the anterior pituitary gland chromophobes are the most common (41%). These cells are small in size, which is on average 8.84 ± 1.12 microns, the cytoplasm does not perceive dyes well, or they surround the nucleus in the form of a transparent rim (c, 3). The nuclei have a round, less often elongated shape, the nucleolus is located in the center. The chromatin apparatus is mainly represented by heterochromatin, which often occupies a large part of the nucleus, while the nucleus looks dark. The core diameter is on average 5.03 ± 0.54 microns. They are located on the entire surface of the anterior pituitary gland, rarely forming small clusters.

Table 1. Morphometric parameters of the cellular composition of the bovine adenohypophysis

Cell types	chromophobes	acidophiles	basophils
Number of cells in %	41%	38%	21%
DC microns	$5,03 \pm 0,54$	$6,79 \pm 0,6$	$7,03 \pm 0,61$
DCell microns	$8,84 \pm 1,12$	$12,31 \pm 0,89$	$11,51 \pm 0,79$
SC micron ²	$16,22 \pm 2,4$	$31,24 \pm 3,63$	$29,24 \pm 4,62$
SCYT micron ²	$26,82 \pm 8,41$	$84,97 \pm 10,22$	$47,32 \pm 8,61$
NCR	0,6	0,59	0,61

Note: DC - diameter core, DCell - diameter cells, SC - core area, SCYT - the area of the cytoplasm, NCR - the nuclear-cytoplasmic ratio

At the ultrastructural level, chromophobes are characterized by small size, light cytoplasm, free of secretory granules, and a rounded nucleus (Fig. 2, b, 4). Clearly visible is the karyolemma, which is adjacent to a large amount of heterochromatin. The granular endoplasmic reticulum (gEPS) is poorly expressed. In the cytoplasm, there are a small number of round-shaped mitochondria and vacuoles without contents. The cell borders are blurred.

Of the chromophilic structures in the anterior pituitary lobe, acidophiles are most common (38%). These are cells of size from 8 to 13 microns, which on average is 12.31 ± 0.89 microns. (Table 1). They are mostly found in round, oval and angular shapes, forming rather large clusters inside the connective tissue strands. Due to the dense arrangement of the cells, they often have an elongated shape. They also occur singly among other cells of the anterior lobe. The cytoplasm is extensive with smooth contours, well painted with acidic dyes in bright red or purple colors. Granularity is often observed in the cytoplasm. The nucleus is almost always located eccentrically adjacent to the cytolemma, sometimes it can occupy a central position (Fig. 1, c, d). The nuclear-cytoplasmic ratio (NCC) is 0.59. The core diameter is larger than that of chromophobes and averages 6.79 ± 0.6 microns. (table.1). The nucleolus is quite large and occupies a central position. The nucleus is dominated by euchromatin, which has the appearance of small grains, so the nucleus is predominantly light, heterochromatin in the form of thin filaments adjacent to the karyolemma forming dark areas.

The cell population of acidophiles is divided into somatotropes that secrete somatotrophic hormone and lactotropes that synthesize prolactin. Somatotropes are ultrastructurally distinguished by clear outlines of the cytoplasm boundaries filled with secretory granules, which are on average 0.351 ± 0.06 microns in diameter. (Table 2). They occur on the entire surface of the anterior pituitary lobe, often forming clusters (Fig. 2 b. 2). The shape of the cells is oval, rounded with clear contours. The average cell diameter is 12.59 ± 1.28 microns. The nucleus occupies a central position in the cell, but may lie eccentrically. The nucleus is light, with one or two nucleoli. The core diameter averages 5.97 ± 0.61 microns. Chromatin is mainly represented by euchromatin, while a

small amount of heterochromatin is present adjacent to the karyolemma. The boundaries of the nucleus are clearly delineated and the gEPS is adjacent to it, on the membranes of which ribosomes are detected in the form of small granules. Among the secretory granules, there is a small content of round-shaped mitochondria. Lactotropes are observed less frequently than somatotropes and are distinguished by large secretory granules in the cytoplasm, the diameter of which reaches 700-800 nm. , which on average is 0.591 ± 0.076 microns. The core is oval in shape, light, and located eccentrically. Often there is one large nucleolus adjacent to the caryolemma. Chromatin is mainly represented by euchromatin. The average diameter of the core is 6.5 ± 0.25 microns.

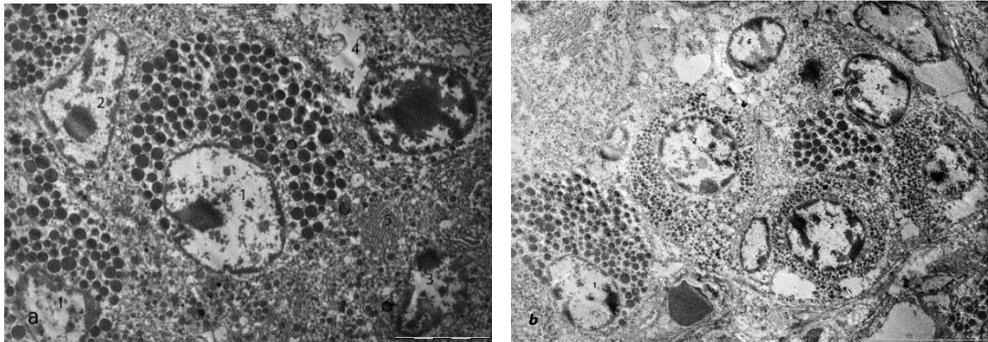


Fig. 2. Electronogram of the anterior pituitary gland of 2 years cattle.
a. 1-lactotrope, 2-thyrotrope, 3-corticotrope, 4-gonadotrope, 5-granular EPS. Uv. x 6000;
b. 1-lactotrope, 2-somatotrope, 3-gonadotrope, 4-chromophobe. Uv. x 6000.

Table 2. Cellular composition of the bovine adenohypophysis

Cell type	Chromophobes	somatotropes	lactotrophs	Gonadotropins	thyrotropics	corticotropes
DC microns	$5,03\pm 0,54$	$5,97\pm 0,61$	$6,5\pm 0,25$	$6,58\pm 0,03$	$5,38\pm 0,79$	$6,28\pm 0,63$
DCell microns	$8,84\pm 1,12$	$12,59\pm 1,28$	$13,24\pm 1,66$	$11,79\pm 0,92$	$11,83\pm 0,68$	$13,8\pm 0,53$
DSG	-	$0,351\pm 0,06$	$0,591\pm 0,076$	$0,265\pm 0,03$	$0,289\pm 0,059$	$0,278\pm 0,024$
SC micron ²	$16,22\pm 2,4$	$27,39\pm 5,026$	$27,26\pm 2,35$	$30,65\pm 1,71$	$17,53\pm 4,35$	$20,23\pm 4,68$
SCYT micron ²	$26,82\pm 8,41$	$54,40\pm 10,31$	$68,47\pm 14,28$	$46,57\pm 2,76$	$45,23\pm 16,44$	$61,86\pm 4,6$
NCR	0,6	0,50	0,39	0,65	0,38	0,32

Note: DC -diameter core, DCell – diameter cells, DSG – diameter of the secretory granules, SC–core area, SCYT – the area of the cytoplasm, NCR– the nuclear-cytoplasmic ratio.

Basophilic adenocytes occupy 21% of the total cell composition of the anterior pituitary gland. There are basophils all over the surface of the adenohypophysis in small clusters, sometimes they can occupy spaces along the entire course of the connective tissue strands forming cells of 30-40 cells. A feature of the location of basophils is the presence of sinusoid capillaries near them. The shape of the cells is often oval, polygonal, less often there are cells with an elongated shape. The cytoplasm Heidenhain's AZAN stain method is painted in blue tones, while the boundaries of the cytoplasm are not always clearly defined and the cell does not always have clear contours. The diameter of basophils is smaller than that of acidophiles, but larger than that of chromophobes and averages 11.51 ± 0.79 microns. In the cytoplasm, there is granularity. The nucleus is large, light has a central position in the cell, less often there are cells with an eccentrically located nucleus. The diameter of the nuclei is on average 7.03 ± 0.61 microns, which is larger than the diameter of the nuclei

in acidophiles and chromophobes. In the center of the nucleus, one or two large nucleoli are visible. Chromatin in the nucleus is mainly represented by more active euchromatin, which is characterized by a uniform distribution around the nucleoli and has the appearance of small granules. Less common are areas of heterochromatin that form small dense areas adjacent to the karyolemma. The nuclear-cytoplasmic ratio in basophils is 0.61.

Among the basophilic cells of the anterior pituitary gland there are gonadotropes that secrete luteinizing and follicle-stimulating hormones, thyrotropics that secrete thyroid-stimulating hormone, and corticotropes that produce adrenocorticotropic hormone.

Gonadotropes occupy the distal part of the anterior lobe of the pituitary gland, but are also found in other parts of the gland. The shape of the cells is often polygonal, oval with clear outlines of the boundaries of the cytoplasm (Fig. 2, a. 4). The nucleus is rounded and has a central position, but may be adjacent to the periphery of the cell. The diameter of the core can vary within 5-7 microns, and is 6.58 ± 0.03 microns. (Table 2). In the cytoplasm, a small number of small granules of the secret are clearly detected, the diameter of which is 0.265 ± 0.03 microns on average. In the cytoplasm, the gEPS localized around the nucleus and the Golgi complex (GC) is well expressed, and there are large number of vacuoles. The NCR– the nuclear-cytoplasmic ratio is 0.65.

Thyriotropes are found in the parenchyma of the anterior pituitary gland less than gonadotropes, whose distinctive feature is their angular elongated shape and small size. They are often adjacent to the sinusoid capillaries. The nucleus is light, angular, less often elongated, the diameter of it 5.38 ± 0.79 microns. There one or two nucleoli occupying a central position. Active euchromatin predominates in the nucleus, and heterochromatin granules form small clusters adjacent to the periphery of the nucleus (Fig.2, a.2). A small number of secretory granules adjacent to the cytolemma can be observed, in the cytoplasm, with an average diameter of 0.351 ± 0.06 microns. The Golgi apparatus is well developed, the gEPS in the form of tubes and membranes is adjacent to the nucleus. Round-shaped mitochondria are often found in various parts of the cytoplasm. NCR– the nuclear-cytoplasmic ratio is less than that of gonadotropins and is 0.38.

Corticotropes have an elongated, often irregular shape with a segmented nucleus. They are located singly among the other cells of the anterior pituitary gland. The cell diameter is on average 13.8 ± 0.53 microns. (Table 2). The nucleus has an irregular, sometimes lobular shape, located in the center of the cell. The average diameter of the nucleus is 6.28 ± 0.63 microns, and heterochromatin and euchromatin are more or less evenly distributed (Fig. 2, a. 3). In the cytoplasm, secretory granules are present in a small amount, diameter of which is 0.278 ± 0.024 microns. In addition to secretory granules, the cytoplasm contains well-developed gEPS, vacuoles, and a large number of round-shaped mitochondria.

4 Conclusion

The studies carried out using histological, morphometric and ultramicroscopic techniques revealed a number of morphofunctional features of the cell composition of the anterior pituitary gland of cattle at a definitive age:

1. The anterior pituitary occupies 64 % of the entire pituitary parenchyma, while the posterior and middle lobes occupy 27% and 9%, respectively.
2. In the anterior lobe of the pituitary gland, using general histological methods, functionally inactive chromophobes (41%) and chromophilic cells, which include acidophils (38%) and basophils (21%), are detected.
3. With the help of electron microscopic studies in the parenchyma of the anterior pituitary lobe, somatotropes are most often found that differ in the presence of a large number of secretory granules in the cytoplasm. Lactotropes are less common than somatotropes and differ from them in larger granules of secretions. Corticotropes, gonadotropes and

thyrotropes with a minimum content of secretory granules are the least detected. All identified endocrine cells are at different stages of functional activity.

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