КРАТКИЕ СООБЩЕНИЯ

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CHANGES IN THE MORPHOMETRIC PARAMETERS OF THE ADRENAL CORTEX OF MARAL DOES DURING THE YEAR = ИЗМЕНЕНИЯ МОРФОМЕТРИЧЕСКИХ ПОКАЗАТЕЛЕЙ КОРКОВОГО ВЕЩЕСТВА НАДПОЧЕЧНИКОВ У САМОК МАРАЛА В ТЕЧЕНИЕ ГОДА

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Изучена гистологическая структура коркового вещества надпочечников холостых половозрелых самок марала в разные сезоны года (на примере животных, проживающих на фермах свободного выгула Республики Алтай). Наибольшая толщина коркового вещества отмечена в зимний период. Минимальная толщина клубочковой зоны характерна для летнего периода, осенью размеры клеток статистически значимо увеличиваются. Пучковая зона зимой расширена, а минимальные ее размеры выявлены в весенний период. Минимальная в году толщина сетчатой зоны наблюдается в зимний период, при этом зимой в ней статистически значимо снижаются размеры клеток и ядер по сравнению с осенью.

Ключевые слова: *корковое вещество надпочечников, клубочковая зона, пучковая зона, сетчатая зона, морфометрия, сезонные изменения, самки марала.*

The histological structure of the endocrine organs of animals has sex-related differences. The adrenal glands, being formed from different embryonic rudiments, produce several hormones that affect periodicity in the body's vital processes. Seasonal changes in the activity of the adrenal glands are noted by many authors [1–4]. In the literature, there are data on sex-related differences in the structural and functional features of deer adrenal glands during the annual cycle [5, 6]. Due to the yearly spring growth of antlers in maral bucks, the structural characteristics of the

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parenchymal cells in their adrenal glands can differ from those in does not only under the influence of factors common to other animals. Changes in the structure of deer adrenal glands in different periods of postnatal ontogenesis have been described in reindeer and roe deer [2, 7]. Seasonal changes in the histological structure of the adrenal glands of marals have been characterized only in bucks and in pregnant does [8, 9].

The aim of this paper was to describe the histological structure of the zona glomerulosa, zona fasciculata, and zona reticularis of the adrenal cortex of non-pregnant maral does in a continuous series of spring, summer, autumn and winter. As the material for the study, we used adrenal glands of healthy sexually mature (5–7 years old) non-pregnant maral does (*Cervus elaphus sibiricus*) living on the free-range farms of the Altai Republic.

The material was obtained from 28 animals: from 7 does in April (1 aged 5 years, 3 aged 6 years, and 3 aged 7 years), from 7 does in June (2 aged 5 years, 3 aged 6 years, and 2 aged 7 years), from 6 does in October (3 aged 6 years and 3 aged 7 years) and from 8 does in December (3 aged 5 years, 3 aged 6 years, and 2 aged 7 years). The procedure was performed in accordance with the requirements of Federal Law no. 52-FZ "On the Animal World" dated 24 April 1995 (edited 7 March 2016) (Articles 34, 35, 40, 44) and with Directive 2010/63/EU. The animals were killed by one shot to the head during planned slaughter. Organ fragments were fixed in 10 % neutral formalin. On the basis of the obtained material, paraffin sections with a thickness of 5–7 μm were made. The sections were stained with haematoxylin and eosin using classical histological techniques. The thickness of the adrenal cortex, the diameter of the cells in all zones of the adrenal cortex, as well as the volume of their nuclei were determined [10]. The nuclear-cytoplasmic (N:C) ratio of the cells was calculated using the following formula:

$$\text{N:C ratio} = d^{2}_{n\ \max}/d^{2}_{c\ \max},$$

where $d^{2}_{n\ \max}$ is the maximum diameter of the cell nucleus and $d^{2}_{c\ \max}$ is the maximum diameter of the cytoplasm of the same cell [10].

To calculate the parameters under study, a MicroMed microscope with Toup Vien software was used. We compared the values of the morphometric parameters obtained from marals in winter with spring, in spring with summer, in summer with autumn, and in autumn with winter to establish continuous changes in the structures of the adrenal glands throughout the year (see Table). A total of 300 measurements of each parameter were made in each season group. Statistical analysis of the results was performed using the software package STATISTICA 10.0 for Windows. The significance of the differences between the groups was determined using the nonparametric Mann–Whitney *U* test. The differences were considered significant at $p < 0.01$.

Maral adrenal glands have a histological structure typical of mammals. The greatest thickness of the adrenal cortex is recorded in winter, which is associated with the maximum size of the zona fasciculata during this season (see Table).

The zona glomerulosa of maral adrenal glands is comprised of small cells that form strands arranged in clusters. In winter, the thickness of the zona glomerulosa is 247.07 ± 11.10 μm. In spring, it remains practically unchanged (see Table). During this period, the blood capillaries expand, the volume of the nuclei increases (Fig. 1), which indicates not only active synthesis, but also excretion of hormones. The minimum thickness of the zona glomerulosa is identified in summer; the capillary network is visually smaller, compared to the previous season group (Fig. 1). In autumn, the thickness of the zona glomerulosa increases due to the growing cell size (cell diameter increases significantly); blood vessels have large gaps, compared to the zona glomerulosa in other season groups.

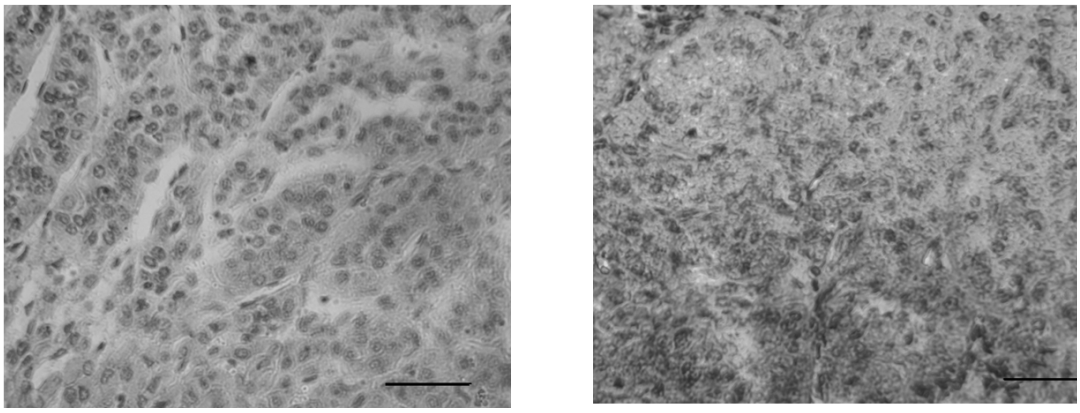
In the zona fasciculata of the adrenal cortex of non-pregnant maral does, the cellular strands have a radial arrangement. The greatest thickness of the zona fasciculata is observed in winter, sharply decreasing in spring, despite the fact that the karyometric parameters show no significant differences between these periods (see Table).

SEASONAL CHANGES IN THE MORPHOMETRIC PARAMETERS OF THE ADRENAL CORTEX OF NON-PREGNANT MARAL DOES, $M \pm \sigma$

СЕЗОННЫЕ ИЗМЕНЕНИЯ МОРФОМЕТРИЧЕСКИХ ПОКАЗАТЕЛЕЙ КОРЫ НАДПОЧЕЧНИКОВ У ХОЛОСТЫХ САМОК МАРАЛА, $M \pm \sigma$

Parameter	Winter (n = 8)	Spring (n = 7)	Summer (n = 7)	Autumn (n = 6)
Thickness of the adrenal cortex, μm	2314.15 ± 89.04***	1408.15 ± 11.00***	1619.84 ± 10.43***	1569.78 ± 10.29***
Thickness of the zona glomerulosa, μm	247.07 ± 11.10	240.22 ± 3.22	235.68 ± 2.55**	247.96 ± 9.50
Diameter of the zona glomerulosa cells, μm	9.13 ± 0.16	9.47 ± 0.73	9.16 ± 1.03**	11.77 ± 1.07
Volume of the nuclei of the zona glomerulosa cells, μm^3	55.31 ± 3.82***	97.36 ± 3.17***	82.65 ± 29.15***	99.07 ± 29.13***
Thickness of the zona fasciculata, μm	1892.59 ± 8.67***	967.17 ± 7.55***	1148.67 ± 8.17	1175.59 ± 10.32***
Diameter of the zona fasciculata cells, μm	10.82 + 1.08	12.49 ± 1.10	12.10 ± 1.29	13.59 ± 1.27
Volume of the nuclei of the zona fasciculata cells, μm^3	84.84 ± 22.16	113.86 ± 12.49	90.07 ± 24.61	97.75 ± 26.70
Thickness of the zona reticularis, μm	174.48 ± 6.77**	200.75 ± 5.19**	235.48 ± 4.21***	208.20 ± 3.54***
Diameter of the zona reticularis cells, μm	10.18 ± 1.02	11.34 ± 1.32	12.04 ± 1.18	13.74 ± 1.14**
Volume of the nuclei of the zona reticularis cells, μm^3	65.46 ± 18.22	81.41 ± 26.49	80.07 ± 24.61	97.75 ± 26.70**

Note. The difference from the subsequent group is statistically significant: ** – when $p < 0.01$, *** – when $p < 0.001$.



Spring Summer

Fig. 1. Zona glomerulosa of the adrenal gland of non-pregnant maral does aged 6 years in spring and summer (stained with haematoxylin and eosin; lens 40, eyepiece 10; scale segment 50 μm)

Рис. 1. Клубочковая зона надпочечника холостых 6-летних самок марала в весенний и летний периоды (окраска гематоксилин-эозином; объектив 40, окуляр 10; масштабный отрезок – 50 мкм)

In summer, the zona fasciculata grows in size significantly, while cell diameter and nuclei remain practically unchanged. The increase in the thickness of the zona fasciculata is due to the growing number of cells. During this period, the lumen of blood capillaries between the strands of cells expands. In autumn, the histological structure and values of the morphometric parameters are similar to those in summer. In winter, the size of the zona fasciculata increases quite sharply, while the morphometric parameters of cells and nuclei remain the same as in autumn. This indicates an increase in the number of cells, since no noticeable changes in the thickness of the capillary network are detected. Thus, we can conclude that the fascicular layer is growing.

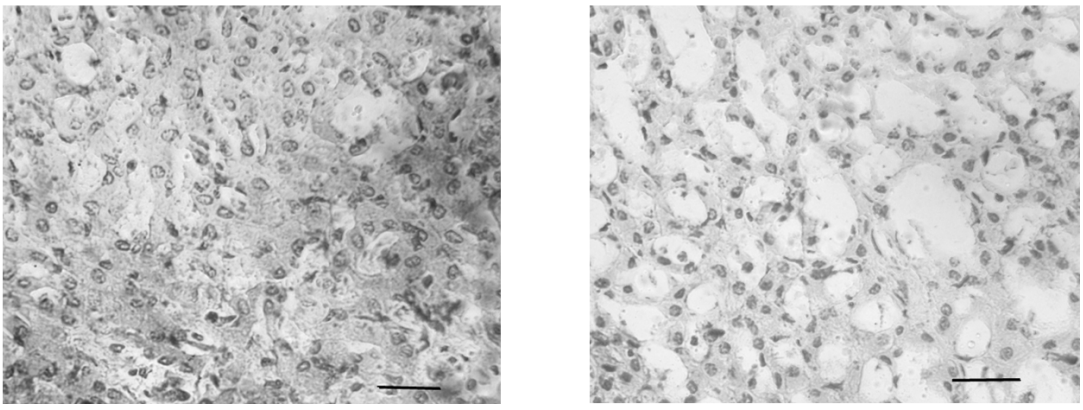
Seasonal changes in the structure of the zona reticularis are similar to those in the fascicular layer in that, out of all the morphometric parameters, only the zone's thickness changes significantly during spring, summer and autumn (see Table). At the same time, as the zone's thickness increases, the network of sinusoidal capillaries expands during the year. The minimum size of the zona reticularis is recorded in winter. Cell diameter and volume of cell nuclei decrease in winter compared to autumn, while the changes do not affect blood vessels (Fig. 2).

There are literature data on sex-related differences in the morphofunctional activity of the adrenal cortex in mammals [6, 11]. The changes in the morphometric parameters we established indicate that the early response to cold described in many mammals is not typical of marals, which is expressed in increased activity of the glandular cells of the adrenal cortex [12].

The expansion of the zona fasciculata in winter due to the growing number of cells correlates with the regeneration processes established in mammals due to the transition zone between the zona glomerulosa and the zona fasciculata [1].

It has been previously established that the histological patterns of adult maral bucks also change during the year [13]. In the zona glomerulosa, bucks show increased values of morphometric parameters in spring (with higher ambient temperatures and the transition to juicier food), while does, in autumn (with lower temperatures and the onset of the sexual reproduction period); in the zona fasciculata, bucks – in spring, does – in summer and winter (coldest season); in the zona reticularis, bucks – in spring (during the growth of antlers) and in autumn (breeding season), while does – only in autumn.

Thus, uneven seasonal changes in the histological structure of different layers of the adrenal cortex of non-pregnant maral does were established. Differences



Autumn Winter

Fig. 2. Zona reticularis of the adrenal gland of non-pregnant maral does aged 6 years in autumn and winter (stained with haematoxylin and eosin; lens 40, eyepiece 10; scale segment 50 μm)

Рис. 2. Сетчатая зона надпочечника холостых 6-летних самок марала в осенний и зимний периоды (окраска гематоксилин-эозином; объектив 40, окуляр 10; масштабный отрезок – 50 мкм)

in the cellular activity of the adrenal cortex in maral does and bucks during different seasons of the year were revealed, indicating sex-related differences in metabolism in the cold winter season, the spring period of active growth of antlers in males and in the autumn period of sexual reproduction.

Conflict of interest. Authors declare no conflict of interest.

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CHANGES IN THE MORPHOMETRIC PARAMETERS OF THE ADRENAL CORTEX OF MARAL DOES DURING THE YEAR

The paper studies the histological structure of the adrenal cortex in non-pregnant sexually mature maral does in different seasons of the year (exemplified by animals living on the free-range farms of the Altai Republic). The greatest thickness of the adrenal cortex is observed during winter.

The minimum thickness of the zona glomerulosa is typical of summer, while in autumn the cell size increases significantly. The zona fasciculata expands during winter, reaching minimum dimensions in spring. The minimum thickness of the zona reticularis is identified in winter; its cells and nuclei decrease statistically significantly in size during this season compared to autumn.

Keywords: *adrenal cortex, zona glomerulosa, zona fasciculata, zona reticularis, morphometry, seasonal changes, maral does.*

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