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RESEARCH ARTICLE

Age-related characterization of endocrine products of Altai maral (*Cervus elaphus sibiricus* Severtzov, 1872): a comparative analysis

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Abstract

The purpose of this study was to evaluate the suitability of different age periods to select endocrine products from red deer by characterizing the morphological and physiological indicators of their testes, adrenals, and thyroid glands. We used classical histological, morphometric, and statistical methods to analyze the functional state of the endocrine glands in red deer aged 1.5 to 2.5 years, 6 to 10 years, and 13 to 18 years. Our findings showed that the morphometric indicators of the functional state of these glands exhibit specific dynamics for each age period. We also analyzed age-related characteristics of the functional state of these glands for the first time in order to identify their potential as additional products in deer breeding. Our results demonstrated that all glands remain functionally active throughout the study age periods and can be used as endocrine raw materials in deer breeding. Furthermore, we did not observe evident destructive changes in the structure of these glands with age, which distinguishes them from other mammalian species, such as reindeer. The preservation of normal functioning of the testes and adrenal cortex may be specific to the technology used to keep deer. When selecting endocrine raw materials for the pharmaceutical industry, our results suggest that the optimal age to collect testes and adrenal glands is between 6 and 10 years, while that of the thyroid gland is 1.5 years. This coincides with the age periods in which the organs exhibit the highest functional activity. In summary, our study provides valuable information on the morphological and physiological characteristics of endocrine products in red deer and identifies the most optimal age periods for their selection. Our analysis demonstrates that the functional activity of these glands varies depending on age and that they remain viable for use in the pharmaceutical industry.

Keywords

Altai maral, red deer, testes, thyroid, adrenal gland, morphological and physiological indicators, structural and functional status, age dynamics, endocrine raw materials, young deer antlers

Introduction

Red deer breeding is an important industry in Russia, particularly the maral subspecies (*Cervus elaphus sibiricus* Severtzov, 1872) in the Altai Krai and Altai Republic since the 19th century. Antlers and other secondary products, including about 30 organs and body parts of deer, are highly valued in both domestic and foreign markets due to the presence of biologically active substances. These products are popular in Southeast Asia, where they are used in combination with medicinal herbs (Lunitsyn, Borisov 2012; Mengyan Li et al. 2018; Chunyi Li 2019).

The study of the morphofunctional state of the testes, adrenal glands, and thyroid glands of red deer is important in determining the optimal age periods for their selection as products containing biologically active substances. The article presents generalized data on the age dynamics of these organs and discusses the issue of expanding the range of secondary raw materials, particularly endocrine raw materials, to increase the profitability of antler reindeer breeding.

Currently, certain types of endocrine enzyme products are obtained from Altai red deer, but the industry needs to increase efficiency through the most complete and wasteless use of all products. To achieve this, a detailed understanding of the structural and functional characteristics of the relevant organs is necessary. The article analyzes the structural and functional parameters of the red deer endocrine glands, depending on age, to substantiate optimal age periods for gland collection.

Organs and body parts of deer, including approximately 30 secondary products, are widely used in Southeast Asian countries in combination with medicinal herbs for their high content of biologically active substances such as amino acids, peptides, lipids, hormones and minerals (Lunitsyn 2012; Belozerskikh & Lunitsyn 2015; G. Ovcharenko & N. Ovcharenko 2018). Shelepov (1998) classified these products into three types: endocrine, enzymatic, and special raw materials. To increase the efficiency and profitability of antler reindeer breeding, it is necessary to expand the use of secondary raw materials, particularly endocrine raw materials, which are not yet processed in antler farms in the Altai region (Maksimov 2019). Currently, Altai red deer are used for certain products of endocrine enzymes such as tails (with a tail gland), testes (with penis), and special raw materials (blood, veins of limbs, embryos), but a more detailed understanding of the structural and functional features of relevant organs is needed (Lunitsyn & Nepriyatel 2016).

The morphological and functional state of endocrine glands in both animals and humans has been studied in depth, including their role in the growth and development of deer, including antlers. Previous research on testes has been conducted by various authors, such as Shevlyuk (1998), Bokova et al. (2014), Bubenic (1990), and Kudryashova (2015). Meanwhile, the adrenal glands were studied by Ryzhavsky (1989), Ovcharenko and Gribanova (2015), and Zolnikova et al. (2019), while the thyroid gland was examined by Bykov (1979), Drzhevetskaya (1994), and Ovcharenko et al. (2016).

Our article takes a novel approach by analyzing the data obtained with a focus on the studied organs as endocrine raw materials. Thus, our study highlights the importance of expanding the range of secondary raw materials, especially endocrine raw materials, in the breeding of antler reindeer in the Altai region. The goal was to characterize the morphophysiological parameters of the testes, adrenal glands, and thyroid glands of red deer for use as endocrine raw materials.

Materials and methods

To study semi-captive marals on reindeer farms in the Altai Republic and the Altai Territory, we collected testes, adrenal glands, and thyroid glands from slaughtered animals during the winter (December-January) when the meat was prepared for consumption. The age of the animals was recorded by checking the inventory books, and we examined a total of 50 glands from 20 individuals. We classified the animals into physiologically grounded age groups using Jaeger and Deev's (1994) principles of animal appraisal. To prepare the specimens, we fixed the glands in a 10% aqueous solution of neutral formalin, Shabadash, and Carnois liquids. We then created paraffin sections that were 4-7 μm thick and stained them with hematoxylin-eosin and the Van Gieson method (Microscopic techniques 1996). The morphological criteria for evaluating gland function were based on the works summarized in the book "Endocrine regulation of growth and development of the body of deer" (2010). Finally, we used Statistica v. 10.0 to perform statistical analysis of the morphometric data.

Result

We conducted a study on maral testes as endocrine raw materials, which are of interest as producers of sex hormones that greatly influence metabolism in the body and the formation of secondary sexual characteristics, particularly horns. To characterize the endocrine activity of maral testes, we considered the following morphological indicators: the weight of the testes, the volume fraction of the interstitium in the total volume of the testes, the number of active Leydig cells per unit area, the volume fraction of active Leydig cells in the volume of the interstitium, and the average volume of their nuclei (see Table 1).
We observed the most significant increase in testicular mass in young animals, with a 2.7-fold increase from 1.5 to 2.5 years of age. However, when reaching maturity, the weight increases only 1.3 times and then stabilizes, demonstrating significant individual variability of this indicator in all age groups. The volume fraction of the interstitium in the total volume of the testes remains stable throughout life, ranging from 18%-23%. The number of active Leydig cells per unit area doubles from 1.5 to adult male deer, and the fractional contribution of Leydig cells to the volume of the interstitium remains constant at 14% to 19% from prepubertal age throughout life, probably a characteristic of the winter season.
The average indices of the volumes of Leydig cell nuclei are minimum in 1.5-year-old animals, slightly but significantly ($P<0.05$) increasing in 2.5-year-old animals, and reached maximum values in adult males. In old red deer, most of the morphological parameters of the functional state of the endocrine part of the gland remain at the level of sexually mature males, except for the volume of Leydig cells, which decreases significantly ($P <0.001$) but still exceeds that of young

individuals.
Therefore, adult animals have significantly larger testicular weights, more active Leydig cells, and larger volumes of their nuclei than young animals. However, even in young animals, the morphological parameters of the functional state of the endocrine part of the gland demonstrate certain activity from 1.5 years.

Table 1. Age dynamics of indicators of red deer testes (Kudryashova & Ovcharenko 2015)

Indicator	Young males (here and then, age, years)		Adult males (age, years)	
	1.5	2.5	6-10	13-18
Testis weight, g	24.9±6.7***	68.0±7.1***	99.2±6.7	93.0±4.2
Volume fraction of interstitium, %	18.6±1.4	18.1±2.1	22.8±1.4	22.4±1.2
Number of active Leydig cells per unit area	6.2±0.5***	8.4±0.5***	12.9±0.7	12.3±1.1
Volume fraction of active Leydig cells in the volume of interstitium, %	18.0±2.3	14.9± 6.7	16.3± 1.4	19.1±2.4
Average volume of Leydig cell nuclei, μm^3	33.0±0.8*	35.5±0.8***	49.8±0.9***	39.0±0.9

Note: Here and then, the difference between the indicators is reliable compared to each subsequent group, *** P<0.001, ** P<0.01, * P<0.05.

The adrenal glands (*Glandula suprarenalis*) are of interest because they produce a variety of steroid hormones, including corticosteroids and adrenaline, which have a multifaceted effect on the vital functions. To evaluate the functional state of the adrenal glands of the red deer, we considered several general morphological indicators, including gland mass, thickness of the cortex and medulla, and cell diameters and volumes (Tables 2 and 3).
We found that the mass of the adrenal glands increases with the growth of the animals, reaching its highest value in sexually mature red deer and remaining relatively unchanged throughout later life. The absolute thickness of cortical and medullary substances gradually increases with age, but relative indicators indicate that the cortical substance predominates over the cerebral substance throughout the entire period of life. The thickness of the cortical zones also increases in each age period.
In young animals, the thickness of the adrenal cortex does not differ significantly from that of sexually mature red deer, but it increases significantly compared to older animals. The adrenal medulla also expands smoothly with age. Overall, our findings provide valuable information on the morphological indicators that characterize the functional state of the red deer adrenal glands at different stages of life.

Table 2. Age dynamics of morphological indicators of the adrenal glands of red deer (Ovcharenko et al. 2010)

Indicator	Young males		Adult males	
	1.5	1.5	6-10	13-18
Mass of gland, g	5.3±0.3	5.5±1.2	7.0±0.9	6.0±0.8
Absolute thickness of the cortex, µm	18.1±124.7	1673.4±104.7	1851.5±147.3	2673.1±114.5***
zona glomerular, µm	257.5±12.8	252.5±10.6	272.02±14.8*	269.43±7.9
zona fasciculata, µm	1113.2±167.1	1187.6±75.5	1371.8±105.7*	2134.9±83.4**
zona reticular, µm	221.3±24.9	233.3±17.36	207.70±26.72	268.75±23.2*
Relative thickness of the cortical substance, %	59.3±1.4	55.2±0.9***	54.6±0.9	61.1±0.7***
Absolute thickness of the medulla, µm	2646.0±183.0	27.0±120.8	3070.0±054.7	3398.5±281.1
Relative thickness of the medulla, %	40.6±2.1	44.7±1.4**	45.3±4.1	38.8±0.8**

Our study of the red deer adrenal gland revealed that the growth of the fascicular zone was responsible for the appearance of the adrenal cortex during the study period (Tables 2, 3). Specifically, in red deer between the ages of 6-10 years, the absolute thickness of the cortex increased due to the glomerular and bundle zones, and the diameter of the cell. Meanwhile, in the glomerular zone, the relative thickness decreased as a result of the advancing growth of the bundle. However, the morphometric parameters of the reticular zone remained unchanged compared to those of young animals.

Table 3. Age-related changes in the morphometric parameters of adrenal cortex cells and red deer (Ovcharenko et al. 2010)

Indicator	Young animals		Adult animals	
	1.5	2.5	6-10	13-18
		Adrenal cortex: zona glomerulosa of the adrenal glands		
Cell diameter, µm	8.89±0.16	8.59±0.16	8.96±0.14	8.64±0.12
Nuclei volume, µm3	67.03±2.47	65.64±2.02	84.40±2.45***	95.75±2.28***
Nuclear-cytoplasmic ratio	0.37±0.02	0.37±0.02	0.35±0.02*	0.40±0.03*
		zona fasciculata of the adrenal cortex		
Cell diameter, µm	12.25±0.55	11.73±0.46	14.24±0.56	14.39±0.59
Nuclei volume, μm^3	93.40±2.63	95.81±2.3	112.74±3.48***	114.98±3.55
Nuclear-cytoplasmic ratio	0.23±0.01	0.24±0.02	0.20±0.02**	0.18±0.02*
		zona reticularis of the adrenal cortex		
Cell diameter, µm	10.07±0.30	10.10±0.32	10.86±0.57	9.00±0.32
Nuclei volume, μm^3	80.96±2.23	85.05±2.47*	85.01±2.24	91.33±2.56*
Nuclear-cytoplasmic ratio	0.33±0.01	0.34±0.02	0.32±0.02**	0.38±0.01
		Adrenal medulla		
A-cell diameter, µm	16.53±0.53	16.33±0.69	17.34±0.67	15.77±0.57*
Nuclei volume of A-cells, μm^3	118.36±5.43	129.94±6.520	138.56±5.58*	126.50±6.05
Nuclear-cytoplasmic ratio (JACS)	0.15±0.01	0.16±0.01	0.14±0.01	0.16±0.01
H-cell diameter, µm	10.02±0.36	10.26±0.28	10.00±0.35	10.86±0.42*
Nuclei volume of H-cells, µm3	111.06±5.39	119.33±5.30	120.41±5.17	113.03±5.56
Nuclear-cytoplasmic ratio (JACS) H cells	0.36±0.02	0.39±0.02	0.37±0.02*	0.32±0.01

In deer over 13 years of age, the absolute thickness of the cortex increased due to the bundle and reticular zones, partly due to the proliferation of intraorganic stromal components and the expansion of the capillary network. The diameter of the cell decreased and the nucleus of the nucleus increased, leading to a decrease in the relative thickness of the glomerular zone.

The diameter of the cells in the glomerular and reticular zones of the adrenal glands decreased, while the volume of their nuclei increased, indicating signs of inhibition of synthetic processes and compensatory reactions in these cells. Furthermore, in the adrenal medulla of stag beetles, the morphometric parameters differed from those of young deer, indicating that the medulla functions in adult animals the same way as during puberty (see Tables 2, 3).

Table 4. Age dynamics of the morphological parameters of the red deer thyroid gland

Indicator	Young animals		Adult animals	
	1.5	2.5	6-10	13-18
Lobus weight, g	4.51±0.43	6.57±0.23*	8.91±1.91*	9.10±0.51
Follicle diameter, μm	136.86±8.95	140.00±9.92**	171.90±6.49**	189.9±8.05
The number of follicles in the field of view	25.5±3.10	29.8±3.30**	14.8±3.10**	11.0±1.60
Thyrocyte height, μm	6.19±0.21	6.18±0.13***	4.78±0.12***	3.73±0.08
Volume of thyrocyte nuclei, μm^3	37.43±2.51	41.20±2.33	46.57±2.80**	30.26±1.88***
Nuclear-cytoplasmic ratio (JACS)	0.57±0.03	0.57±0.04	0.58±0.01	0.56±0.05
Parenchyma to stroma ratio in the gland (PSC)	13.67±1.69	13.75±1.28	10.19±0.79***	8.70±0.69***
Brown Index	21.11	22.45	34.93	49.86

When it comes to the thyroid gland, it is of interest primarily due to its production of the iodine-containing hormones thyroxine and triiodothyronine, as well as the non-iodized hormone thyrocalcitonin. These hormones play a crucial role in the regulation of all types of metabolism. In our study, we considered several morphological indicators to characterize the functional state of the maral thyroid gland, including mass, diameter of the follicles, number of follicles in the field of the view, height of thyroid cell, volume of nuclei, NCC (nuclear-cytoplasmic ratio), PSC (parenchyma and stroma) and Brown index. We also take into account the ratio of large, medium, and small follicles in the gland.

Our findings showed that the mass of the thyroid gland in marals increased with age, but the functional activity of the gland was higher in young red deer, from one and a half years of age. This was evidenced by the smallest average diameter of the follicles, the maximum frequency of occurrence of small follicles (80%-83%), the minimum values of the Brown index and the maximum height of thyroid cells, and the volume of their nuclei. In contrast, in adult stags, there was a reduction in thyroid activity compared to young ones. This was confirmed by a significant increase in the average diameter of the follicles, a sharp decrease in the proportion of small follicles (40%), and an increase in the proportion of medium and large follicles (32% and 28%, respectively). Thyrocyte height decreased with age and the volume of their nuclei increased. Brown's index values also increased, and the average diameter of the gland follicles reached its maximum value in the age range, with the number of small follicles being minimal and the number of large ones being maximal (30%).

Table 5. Percentage of follicles in the thyroid gland of red deer by age (Ovcharenko et al. 2010)

Indicator		Young animals		Adult animals	
		1.5	2.5	6-10	13-18
Small folicles	Diameter, µm	68.88±4.51	72.36±4.53	85.32±6.89	100.00±6.08
	Frequency, %	80±3.0	83±3.0	40±3.0	30±6.0
moderate f.	Diameter, µm	119.01±3.88	133.27±8.22	172.58±8.52	172.78±7.95
	Frequency, %	12±2.0	12±2.0	32±3.0	39±2.0
Large f.	Diameter, µm	222.91±17.45	214.92±13.78	265.68±12.67	295.92±14.96
	Frequency, %	7±2.0	5±2.0	28±2.0	31±1.0
In the field of vision, total		25.53±3.12	29.87±3.10	16.80±3.10	11.01±1.60

Observations reveal a decrease in the number of interfollicular cells, while large follicles are dominated by the squamous epithelium, leading to the Brown index reaching its maximum value of 49.86 within the given age range. Furthermore, the parenchyma-stromal coefficient decreases significantly compared to the previous group, indicating a decrease in the parenchyma due to the growth of stromal elements. These structural changes in the thyroid gland are compensatory in sexually mature males and more pronounced in aging animals, along with other signs of aging.

Discussion

Our results showed that there were specific gland rearrangements for each age group. Consistent with observations in other mammalian species, we found that the mass of the testes increased with age (Shevlyuk et al., 2010). Additionally, we observed that Leydig cells in adult male red deer had the highest functional activity, as confirmed by the age dynamics of testosterone in peripheral blood (Lunitsyn et al., 2003). Although the morphological parameters of endocrine activity in the testes of older males were similar to those of adult males, the volume of Leydig cell nuclei differed. We attributed this difference to the season and the peculiarities of animal culling, since the glands were taken during a period of sexual dormancy (winter), when their functional activity is generally reduced (Kudryashova and Ovcharenko, 2015). Furthermore, deer herding farms culled animals with antler products that did not meet the standard, which typically occurred between the ages of 12 and 14 (Jaeger and Deev, 1994). Thus, we hypothesized that old red deer with a level of the functional activity of testes sufficient for the formation of high quality antlers were not subjected to culling. This is because there is a close relationship between the reproductive activity of males and the quality of antlers (Lunitsyn and Borisov, 2012), and there is the possibility of preserving antler quality in old age (Khmelnitsky and Stupina, 1989).

Our study also found that the mass of adrenal glands increased during the growth of the animal, consistent with observations in other mammalian species (Kozlo 1983; Sidorov 1987; Drzhevetskaya 1994; Shishkin 1997). In marals, differentiation and growth of the cortical and medulla occur in the initial stages of ontogenesis, along with the formation of a connective tissue structure and the development of the vascular bed. The functional state of the cortex reaches its highest point in red deer between the ages of 6 and 10 years, and conflicting information exists in the literature on the functional state of adrenal glands in mammals during aging (Ovcharenko et al. 2015). In our study, we found a slight decrease in the functional state of the adrenal glands in old red deer, although absolute indicators of both the cortex and the medulla were the highest in this age group. In marals, we observed that the synthetic activity of cells of the reticular layer decreases at the age of 13-18 years, and the increase in the thickness of the zone and the volume of cell nuclei during this period can be considered a compensatory response to other changes in cell structure. However, Ryzhavsky (1976) reported that in humans the thickness of the reticular zone does not change with old age and an increase in the size of cells occurs. In humans and laboratory animals, the volume of cell nuclei in old age decreases (Khmelnitsky, Stupina 1989).

Furthermore, our study found significant changes in the morphological parameters of the red deer thyroid gland with age. In young animals, the gland has a well-developed capsule, lobulation, and follicular structure. With age, the gland becomes more uniform in structure, the capsule is less pronounced, and the size and number of follicles decrease. These changes may reflect the decrease in functional activity of the thyroid gland with age.

In summary, our study provides new insight into the structural and functional changes in the peripheral endocrine glands of red deer during different age periods. Our findings suggest that the observed changes in the testes, adrenal glands, and thyroid gland may reflect age-related changes in hormonal regulation in red deer. These results may have important implications for understanding the reproductive and endocrine physiology of red deer and may be useful for wildlife management and conservation efforts.

Conclusions

Our study aimed to evaluate the structural and functional state of the endocrine glands in red deer over various age periods by conducting an analysis of morphophysiological indicators. Our findings demonstrate that the testes, adrenal glands, and thyroid glands exhibit functional activity as early as 1.5 years of age and maintain it to varying degrees even in older animals that have surpassed their productive age. Therefore, we recommend selecting endocrine raw materials from red deer of all age groups during the planned mass slaughter in winter. Our observations reveal that these glands in red deer exhibit remarkable resistance to destructive changes in their structure, making them apart from other mammals, particularly reindeer. This may be due to the technology used to keep them, including feeding in parks and selective breeding. We found that the age periods of the animals coincide with the highest functional activity of their glands, making them optimal for selecting endocrine raw materials for use in the pharmaceutical industry. For example, the thyroid gland is best selected at 1.5 years, while the testes and adrenal glands are optimal between 6-10 years. However, we acknowledge the need for further research to explore the biochemical composition of the endocrine organs used as raw materials, which contain biologically active substances. In conclusion, our study provides valuable information on the endocrine glands of red deer, emphasizing their viability as a source of endocrine raw materials. We recommend selecting these raw materials across all age groups during planned mass slaughter and further exploring their biochemical composition to realize their full potential.

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