

Morphofunctional changes in the thyroid gland and pituitary thyrotropes in rams of the Russian long-haired breed in different seasons of the year

Andrey Grigoryevich *Ulyanov*^{1,*}, Natalia Anatolyevna *Slesarenko*², Pyotr Makarovich *Torgun*¹

¹ Voronezh State Agricultural University named after Emperor Peter I, Voronezh, Russia

² Moscow State Academy of Veterinary Medicine and Biotechnology-K. I. Scriabin MBA, Moscow, Russia

Abstract. In mature rams, the signs of high functional activity of the thyroid gland are observed in the autumn period. An increase in the diameter of the thyroid follicles (109.1 ± 0.58 microns) is marked, the height of the epithelium (9.6 ± 0.11 microns) and the area of the thyrocyte nuclei ($32.8 \pm 0.38 \mu\text{m}^2$) increase statistically significant ($P < 0.001$). In the pituitary gland of rams in the autumn period, there is an increase in the number of thyrotropes. The cytoplasm of thyrotropes is filled with secretory granules, which reflects the high level of their functional activity.

1 Introduction

Seasonal changes in the morphofunctional parameters of the thyroid gland are studied in many mammals [1-9]. However, there is no information in the literature about changes in the secretory activity of the thyroid gland in different seasons of the year in rams of the Russian long-haired breed.

The aim of this research is to study the morphofunctional state of the thyroid gland and pituitary gland in rams of the Russian long-haired breed in different seasons of the year.

2 Methods and materials

The material from 30 rams of the Russian long-haired breed was studied. For light microscopy, fragments of the thyroid gland were recorded in the fluids of Shtieve, Buen, and Tsenker. Serial paraffin sections were stained with azan according to Heydenhein, the periodic acid Schiff reaction and the trichrome-Schiff and tetrachrome-Schiff colouring were used. Using a screw eyepiece micrometer, the diameter of the thyrocyte nuclei and the height of the thyrocytes in the thyroid gland were measured (100 measurements for each animal). The area of the thyrocyte nuclei was determined by the diameter of the nuclei. In addition, the diameter of the thyroid follicles was measured (50 measurements for each animal).

In the pituitary gland, the average number of thyrotropes in one visual field was determined at an increase of $\times 1350$ (30 visual fields were used for each animal). The diameter of the pituitary thyrocyte nuclei

was also measured using a screw eyepiece micrometer (100 measurements for each animal). The area of the thyrotropes' nuclei was determined by the diameter of the nuclei. The cytoplasmic area of thyrotropes was determined planimetrically (50 measurements for each animal).

The results of measurements of the area of the thyrocyte nuclei, the area of the nuclei and the cytoplasm of the thyrotropes and the height of the epithelium of the thyroid follicles had a normal distribution and equality of variances was revealed in the comparison groups, and therefore in these cases we applied the Student's t-test. The differences between the average values of the compared groups were considered statistically significant at a significance level of $P < 0.001$. The results of measurements of the diameter of thyroid follicles and the number of pituitary thyrotropes were processed using the nonparametric White T-test. The differences between the average indicators of the compared groups were considered statistically significant at a significance level of $P < 0,05$ [10, 11].

For electron microscopy, pituitary samples were fixed in 2.5% glutaraldehyde on 0.1 M S-collidine buffer at $\text{pH} = 7.3$. The subsequent fixation of the material was carried out in a 1% osmium fixator. The material was dehydrated in acetone of increasing strength and enclosed in epon-812 epoxy resin. The sections were prepared on the BS-490 and LKB-4800 ultramicrotomes. The obtained sections were contrasted with uranyl acetate and lead citrate, and studied using the electron microscope "Tesla BS-500".

*Corresponding author: agu16@mail.ru

3 Results

The results of morphometric studies of the thyroid gland in rams in different seasons and years are presented in table 1.

In rams in the summer (table1, fig.1), the thyroid follicles have a small diameter. The epithelium of the follicles is cubic, the nuclei are rounded or slightly oval. Destructive changes are found in individual thyrocytes. In these cells, the nuclei are dark pycnotic, the cytoplasm is dense and homogeneous. There are no vacuoles in the colloid. All these signs reflect a reduced level of secretory activity of the gland.

In the autumn period, there is a statistically significant increase ($P < 0.001$) in the area of the

thyrocyte nuclei and the height of the epithelium (table1, fig.2) compared to the summer period. Thyrocytes have a high prismatic shape, the nuclei are rounded or slightly oval. The colloid contains numerous small and large vacuoles. There is also a statistically significant ($P < 0.05$) increase in the diameter of the follicles.

These changes indicate an increase in hormone-forming processes in the thyroid gland. There are no thyrocytes with signs of destruction. In winter, rams showed signs of a decrease in the secretory activity of thyroid follicles: the area of thyrocyte nuclei significantly decreased by 12.3% compared to the autumn period (table 1). The height of the epithelium and the diameter of the follicles also decreases by 8.7% and 23.5%, respectively.

Table 1. Morphometric parameters of the thyroid gland in rams in different seasons of the year

Season of the year	n	Area of thyrocyte nuclei (μm^2)	Height of thyrocytes (μm)	Diameter of follicles (μm)
Summer	10	27.46±0.37	7.4±0.12	72.7±0.72
Autumn	10	32.80±0.38*	9.6±0.11*	109.1±0.58**
Winter	10	28.38±0.37*	7.8±0.12*	83.4±0.87

* $P < 0,001$ (Student's t-test); ** $P < 0,05$ (White T-test)

Based on the special and electron microscopic studies conducted in the anterior pituitary lobe of Russian long-haired rams, an increase in the number of tyrotropes in the autumn period was more than twice as large as in the summer period (Table 2). There was also an increase in the area of the nuclei and cytoplasm of the pituitary tyrotropic cells by 21.9 and 25.4%, respectively. When

processing sections using the trichromatic-Schiff method, tyrotropes are found in the largest number in the central part of the pituitary gland. These cells (fig.3) have a polygonal shape, characterized by a weak Schiff-positive cytoplasm, the nuclei are located in the center of the cells.

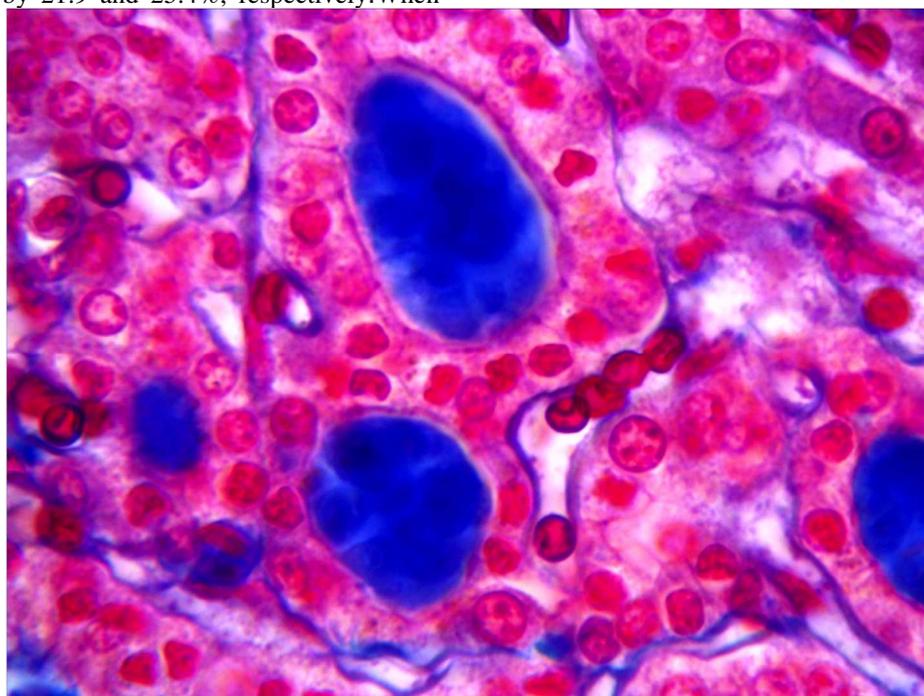


Fig. 1. The thyroid gland of a ram. June. The epithelium of the follicles is cubic, the nuclei are rounded. In the epithelium, there are thyrocytes with dark pycnotic nuclei. There are no vacuoles in the colloid. Fixation: Shtieve's liquid. Colour: Azan according to Heydenhein. Lens magnification x 40, oc. x15.

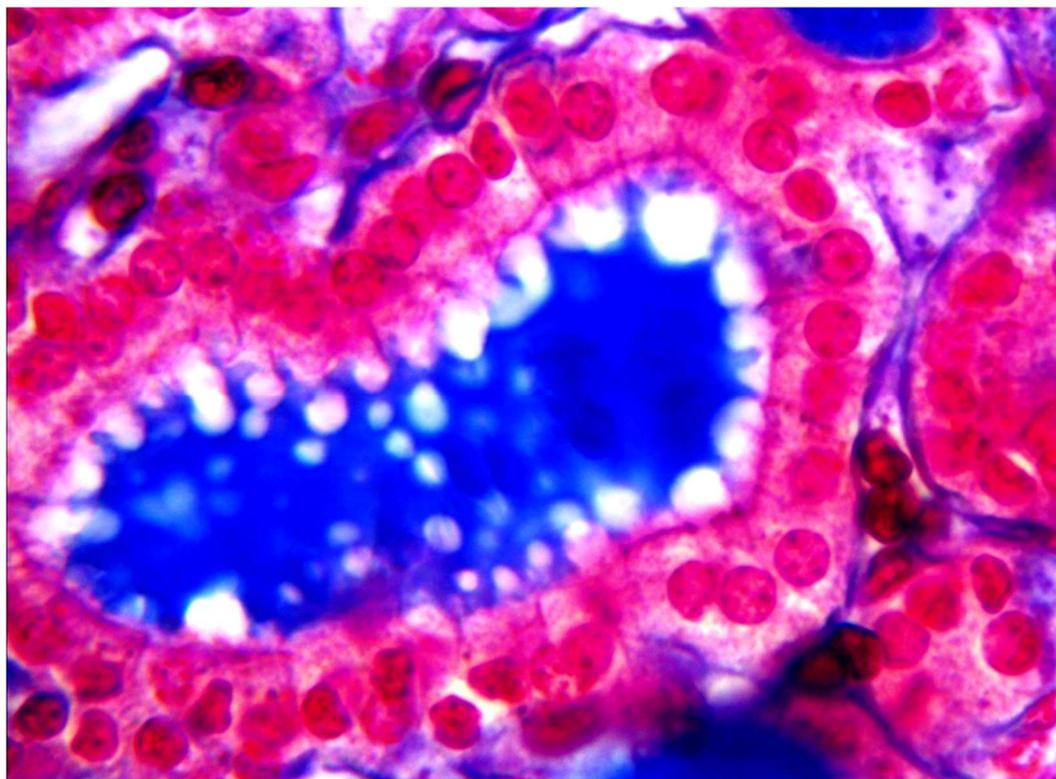


Fig. 2. The thyroid gland of a ram. October. Thyrocytes have a high prismatic shape, the nuclei are rounded or slightly oval. The colloid contains numerous small and large vacuoles. Fixation: Shtieve’s liquid. Colour: Azan according to Heydenhein. Lens magnification x 40, oc. x15.

Table 2. Cytokaryometric parameters of pituitary thyrotropes in rams in different seasons of the year

Season of the year	n	The number of thyrotropes in one field of view	The area of the cytoplasm of the thyrotropes (μm^2)	Area of thyrotrope nuclei (μm^2)
Summer	10	6.3 ± 0.26	51.8 ± 0.42	29.26 ± 0.37
Autumn	10	$13.3 \pm 0.33^{**}$	$64.9 \pm 0.41^*$	$35.65 \pm 0.38^*$
Winter	10	$9.4 \pm 0.42^{**}$	$56.8 \pm 0.43^*$	$30.28 \pm 0.37^*$

* $P < 0,001$ (Student's t-test); ** $P < 0,05$ (White T-test)

In winter, there is a decrease in the number of thyrotropes by 29.3% compared to the autumn period. The area of the nuclei and cytoplasm of thyrotropes also decreases by 12.4 and 15.1%, respectively ($P < 0.001$). These changes reflect a decrease in the secretory activity of the pituitary thyrotropes in the winter period compared to the autumn period.

Electron microscopic studies have shown (Fig. 4) that the thyrotropes in the pituitary gland of rams of the Russian long-haired breed are characterized by a polygonal shape with a diffuse arrangement of small secretory granules. The granules are contained in a small amount and are distributed evenly throughout the

cytoplasm. The electron density of secretory granules varies significantly. In the autumn period, an increase in the number of thyrotropic cells was found in the pituitary gland, both in the central part of the pituitary gland and in the lateral zones. In the cytoplasm of these cells, there is a significant number of small secretory granules, the diameter of which varies from 180 to 230 nm. Well-developed organelles were also identified. Some thyrotropes are characterized by a well-defined degranulated cytoplasm. All these changes reflect an increased level of secretory activity of the pituitary thyrotropes in the autumn period.

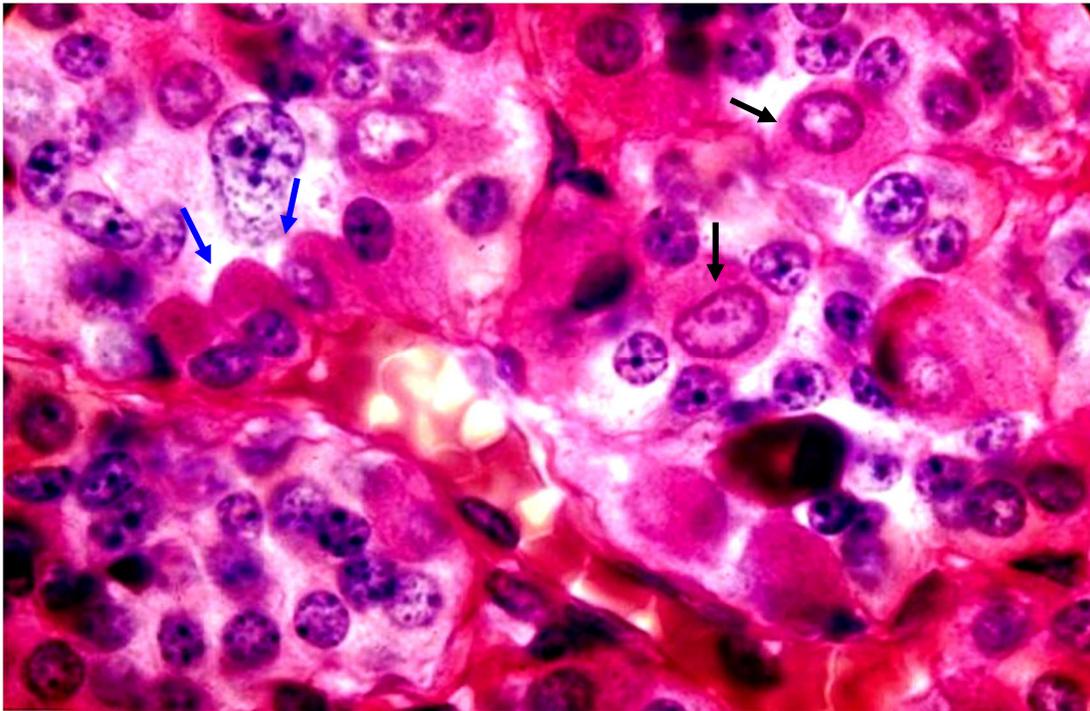


Fig. 3. The pituitary gland of a ram. October. Hypertrophied thyrotropic cells are visible (marked with black arrows). Near the blood vessel on the left are several gonadotropic cells (blue arrows). Gonadotropic cells give an intense Schiff -positive reaction, have an oval shape and eccentrically arranged nuclei. Fixation: Shtieve's liquid. Colour: trichrome-Schiff. Lens magnification is x 90, oc. x15.

4 Conclusion

In conclusion, it can be noted that the morphofunctional parameters of the thyroid gland in rams of the Russian long-haired breed undergo seasonal changes. The highest secretory activity of the thyroid gland in these animals

was founded out in the autumn period, which is confirmed by an increase in the diameter of the follicles, the height of the thyrocytes and the area of their nuclei. In the anterior lobe of the pituitary gland in rams in the autumn period, tyrotropes with signs of high secretory activity are defined.

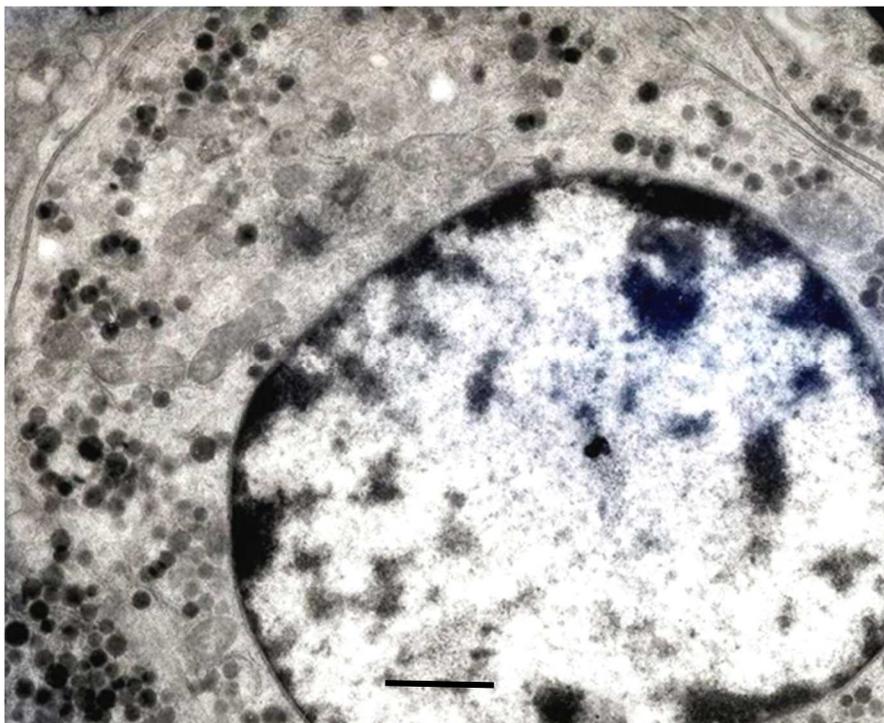


Fig. 4. Electron microscopy of a thyroid-stimulating cell. (Scale = 1 μ m). The cytoplasm contains small amounts of small secretory granules. There are rounded or elongated mitochondria.

References

1. A.Afanasyeva, Functional activity of the endocrine glands during adaptation to seasonal factors in goats of the Gorno-Altai down breed, *Bulletin of Altai State Agrarian University*, **1(17)**, 95-97 (2014)
2. I. Antipin, Age and seasonal changes in the level of thyroid hormones in the blood serum of reindeer of the Bolshezemelskaya tundra, *Mater. 13 Komi Rep. young. scientific Conference*, p. 86(Syktyvkar,1997)
3. V. Kotarev, A. Ulyanov, P. Torgun, Histochemical and electron microscopic studies of somatotropic, tyrotropic and chromophobic pituitary endocrinocytes in different seasons of the year, *Journal of Anatomy and Histopathology*, **3 (4)**, 39-42 (2014)
4. A. Ptashkin, A. Li, Seasonal changes in the functional activity of the thyroid gland in Karakul sheep, *Proceedings of the Institute of Karakul Breeding*, **13**, 407-416 (1961)
5. N. Trush, D. Kleikova, Pedigree and seasonal morphofunctional features of the thyroid gland of cattle of the Amur region, *Agrarian Bulletin of the Urals*, **5(59)**, 77-78 (2009)
6. Xiao-Tuan Liu, Qing-Fen Li, Chen-Xi Huang, Ru-Yong Sun, Changes in peripheral thyroid hormone levels in the ground squirrel *Spermophilus dauricus* during exposure to cold, hibernation, and awakening, *Dongwuxuebao*, **5(47)**, 502-507(2001)
7. Xiao-Tuan Liu, Qing-Fen Li, Chen-Xi Huang, Ru-Yong Sun, Changes in thyroid hormone in the Mongolian gerbil (*Meriones unguiculatus*) when exposed to cold, *Shoulei xuebao*, **2(21)**, 132-136 (2001)
8. P. Nieminen, J. Asikainen, H. Hyvarinen, Effects of seasonality and fasting on the plasma leptin and thyroxin levels of the raccoon dog (*Nyctereutes procyonoides*) and the blue fox (*Alopex lagopus*), *J. Exp. Zool.*, **2(289)**, 109-118(2001)
9. Yongqiang Tian, Xingxu Zhao, Minqiang Wang, Zhonglin Lu, Rongchang Zhang, Endocrine changes and their relationship with body weight in growing yaks, *Anim. Sci.*, **1(74)**, 89-94 (2002)
10. G. Lakin, *Biometrics* (Moscow, 1990)
11. V. Urbach, *Biometric methods* (Moscow, 1964)