Dynamics of morphofunctional activity of blood lymphocytes of Stavropol breed sheep in postnatal ontogenesis and during pregnancy

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Abstract. The aim of the paper was to study the activity of nucleolar organizer zones of blood lymphocytes of Stavropol breed sheep in postnatal ontogenesis and during pregnancy. Blood smears for cytomorphology studies were painted with silver nitrate and subsequent additional coloring with azure and eosin. By measuring the parameters of the nucleus and parameters of the activity of nucleolar organizers (AgNORs), the changes in the morphofunctional state of the protein-synthesizing apparatus of blood lymphocytes in sheep of Stavropol breed in postnatal ontogenesis were evaluated. It was found that newborn lambs' area of the nuclei of lymphocytes was 76.50±2.30 µm². By the age of three months, the area of lymphocyte nuclei in sheep significantly increased by 6.08% and amounted to 81.46±2.45 µm², and did not change significantly until the age of twelve months. The number of units of argentophilic nucleolar organizers in the nuclei of lymphocytes (AgNORs) is from 1 to 4. The average area of AgNORs in the nuclei of lymphocytes is in the range from 73.60±2.45 up to 80.10±2.41 µm². In sheep of Stavropol breed on the 90th day of pregnancy, the area of the nucleus of lymphocytes was 68.70±2.15 µm². By 135 days of pregnancy, a significant decrease in the area of the nuclei of the lymphocytes by 14.66% was found. After childbirth, there was a slight decrease in the area of lymphocytes in sheep compared to the previous period of research. During pregnancy, sheep were found to have from 1 to 3 AgNORs with a total area from 0.70±0.11 to 2.93±0.43 µm². On the basis of the conducted studies, it can be concluded that in postnatal ontogenesis and during pregnancy of sheep, the functional state of lymphocytes changes, this is manifested in changing the parameters of the area of their nuclei, the number and total area of AgNORs.

1. Introduction
The actual direction of determining the morphofunctional state of blood cells in biology in recent years is to study the activity of nucleolar organizer zones (AgNORs). Nucleolus is a specialized structure of eukaryotic nuclei, the main role of which is the synthesis of ribosomal RNA and the assembly of ribosomal units. The following components are distinguished in the nucleolus at the ultrastructural level: fibrillar centers (FC), dense fibrillar component (DFC), granular component (GC), nucleolar vacuoles, and also, associated with the nucleolus, chromatin [1, 2, 3].
According to many domestic and foreign scientists, leukocytes not only protect the body from foreign agents, but also carry out the operational regulation of morphogenesis, supporting the course of normal growth and development of tissues.

It is known that lymphocytes are the most complex functionally active blood cells of mammals, which is determined by the presence of the nucleus in it and providing a number of processes of vital activity of the organism.

Lymphocyte, taking signals of any changes in the internal environment of the body, modulates its functions, taking into account the restoration of general homeostasis. This principle lies at the basis of the response of immune cells, making them universal indicators of norm and pathology [4].

While the total area of the nucleus in the cell increases the activity of nucleolar organizers areas and gene expression [5].

It is known that the nucleolus, being an obligatory organelle of eukaryotic cells, is formed as a result of the transcriptional activity of the main ribosomal genes encoding 18S - 28S - and 5.8 S-classes of rRNA. The nucleolus contains a large number of different types of proteins, some are not directly related to the main function of the nucleolus – the synthesis of ribosomal units [6].

Argentophile nonhistone acidic proteins of the nucleolus (C23, B23, UBF and RNA polymerase I) responsible for the activation and control of transcription of ribosomal genes, are found in the color of black dots (granules) - this is the place where the silver contacted in a cage with acidic proteins relevant to transcription and transformation rRNA [7]. The content of silver granules in nucleoli, as a rule, corresponds to the amount of RNA polymerase I actively working in them. With the help of silver nitrate in the nuclei of cells, it is possible to identify the regions of nucleolar organizers, and by their number and area to judge the intensity of protein synthesis [8,9].

At the light level after silver painting, argentophilic proteins are detected in the interphase only in nucleoli, and during mitosis in the region of the nucleolar organizer of mitotic chromosomes and in perichromosomal material. At the electron microscopic level, these proteins are found in the fibrillar centers and dense fibrillar component, but sometimes in the granular. The drugs dispersed nucleoli typical for them is localized in the transcribed part of DNA matrix on the distributor RI-the fibrils, and the absence in areas retranscribing spacers. This specific distribution of proteins is associated with it participation in the transcription of ribosomal genes [10].

There is insufficient information about changes in the parameters of nucleolar organizer zones in mammalian blood lymphocytes in postnatal ontogenesis in the scientific literature.

In this regard, the aim of this study was to study the parameters of the activity of nucleolar organizer zones of blood lymphocytes of sheep of Stavropol breed in postnatal ontogenesis and during pregnancy.

2. Materials and methods of the studies

Experimental studies were conducted from 2016 to 2018 in sheep farms located in the eastern zone of the Stavropol region, in the clinic of the Department of physiology, surgery and obstetrics and in the “Scientific diagnostic and medical-veterinary center” of the Federal State Budget Education University of high education “Stavropol State Agrarian University”.

The object of the study was the blood of female sheep of Stavropol breed of the following age groups - newborns (up to 7 days), 3, 6, 9, 12 months of life, as well as non-pregnant sheep, during pregnancy (on the 90th day, 135 days) and after childbirth. There were 10 animals in each experimental group.

Blood samples from sheep were obtained in the morning, before receiving feed, from the jugular vein in polypropylene tubes (S-Monovette®, manufactured by SARSTEDT, Germany.) containing an anticoagulant that prevents blood clotting. Blood samples were used to make smears.

Blood smears for cytomorphological studies were painted with silver nitrate by W. Howell and D. Black (1980) in modification of V. I. Truhachev with co-authors (2015). The blood smears were fixed with methyl alcohol, dried and washed with distilled water. Then smears were placed in a solution of KCl (0.57 g KCl per 100 ml of distilled H2O) for 20 minutes, and after washing with distilled water - in a mixture of 50% solution of silver nitrate (solution “A”) and 2% solution of gelatin on 1% solution
of formic acid solutions (solution “B”) prepared by extemore. The solution “A” (5 ml) and “B” (5 ml) were mixed in the dark and in the resulting mixture the blood smears were kept in a thermostat for 20 minutes in the dark at a temperature of 370 C. After that, the smears were immersed for 2-3 seconds in distilled water, held twice for 8 minutes in a 5% solution of thiosulfate Na, (in the dark at 370C), washed with tap water, and then distilled water. To differentiate the blood elements, Romanovsky method of staining was carried out for 5 minutes, washed with tap water, dried and smears were enclosed in a canadian balm.

Painted blood smears were examined using a light microscope OLYMPUS-BX43 (Japan), digital images were obtained using the camera OLYMPUS C 300 (Japan). On each is painted a smear of blood was taking pictures at 10 randomly selected fields of view using a lens of 40× (for observation purposes) and 100× (for morphological studies). Digital images were used to study such parameters as the area of the lymphocyte nucleus, the number and area of nucleolar organizer regions (in 10 nuclei in each image, a total of 100 AgNORs measurements in the smear).

Morphometric studies were performed using the program VideoTesT Master 4.0 for Windows XP (JSC “ISTA”, St. Petersburg) on IBM-compatible computer.

The study materials were analyzed and numerical indicators were processed by the method of one-factor analysis of variance and two-sided Student test in the program Primer of Biostatics 4-03 for Windows. Differences were considered significant at p<0.05.

Used reagents: potassium chloride and sodium thiosulfate (REACHIM, Russia); gelatin (moschympharmreparaty, Russia); silver nitrate (industrial non-ferrous metal plant secondary metal (vtormet), Russia); methyl alcohol (Methanol, Russia); formic acid (Reactiv, Russia); Romanovsky dye (BioVitrum, Russia); canadian balm (Bio-Optica, Italy).

3. Results

The parameters of nuclei, the number of nucleolar organizer zones and their total area in the lymphocytes of sheep of Stavropol breed in postnatal ontogenesis were studied on fixed and painted blood smears (figure -1, 2, 3).

It was found that the lymphocyte area was 76.50±2.30 μm² in the material selected for histochemical examination in newborn sheep (table - 1).

![Figure 1](image_url)

Figure 1. Lymphocyte with argentophilic nucleolar organizer. Newborn. Painting with silver nitrate with painting by the method of Romanovsky. The scale ×400.
By the age of three months, the area of lymphocytes in sheep significantly increased by 6.50% and amounted to $81.46 \pm 2.45 \, \mu m^2$.

From the age of three months to the age of nine months there were no significant changes in the lymphocyte area in sheep of Stavropol breed, but there was a decrease in dynamics (table – 1).

![Figure 2. Lymphocyte with argentophilic nucleolar organizer. 6 month-old. Painting with silver nitrate with painting by the method of Romanovsky. The scale ×400.](image1)

![Figure 3. Lymphocyte with argentophilic nucleolar organizer. 9 month-old. Painting with silver nitrate with painting by the method of Romanovsky. The scale ×400.](image2)
An increase in the area of lymphocyte nuclei was found in twelve-month-old sheep, compared with the previous study period (for 8.11%, p≤0.05).

**Table 1.** Morphological parameters of sheep blood lymphocytes in postnatal ontogenesis.

| Age              | Area of lymphocytes nucleus, µm² (M±m) | Number of AgNORs, units (M±m) | Summary area of AgNORs, µm² (M±m) |
|------------------|----------------------------------------|-------------------------------|-----------------------------------|
| Newborn (n=10)   | 76.50±2.30                             | 2.90±0.16                     | 1.81±0.31                         |
| 3 months-old (n=10) | 81.46±2.45*                           | 1.60±0.14*                    | 1.98±0.23                         |
| 6 month-old (n=10) | 79.25±1.98                             | 4.00±0.24*                    | 4.69±0.86*                        |
| 9 month-old (n=10) | 73.60±2.45                             | 2.00±0.09*                    | 2.48±0.28                         |
| 12 month-old (n=10) | 80.10±2.41*                           | 1.20±0.11*                    | 0.97±0.12*                        |

Note: * - the differences are significant in comparison with the previous period, p≤0.05.

Studies have found that pregnant animals (figure – 4, 5), a slightly different dynamics in the change in the mean area of lymphocyte nuclei was observed (table – 2).

![Figure 4. Lymphocyte with argentophilic nucleolar organizer. 90th day of pregnancy. Painting with silver nitrate with painting by the method of Romanovsky. The scale ×400.](image-url)
Figure 5. Lymphocyte with argentophilic nucleolar organizer. 135th day of pregnancy. Painting with silver nitrate with painting by the method of Romanovsky. The scale ×400.

Table 2. Morphological parameters of indicators of blood lymphocytes of sheep.

| Day of pregnancy | Area of lymphocytes nucleus, µm² (M±m) | Number of AgNORs, units (M±m) | Summary area of AgNORs, µm² (M±m) |
|------------------|----------------------------------------|-------------------------------|----------------------------------|
| Non pregnancy (n=10) | 82.50±2.41 | 1.90±0.11 | 1.01±0.15 |
| 90th day (n=10) | 68.70±2.15* | 1.40±0.09* | 0.70±0.11 |
| 135th day (n=10) | 80.50±2.28* | 1.60±0.11 | 0.78±0.08 |
| Afterbirth (n=10) | 79.50±2.28 | 2.60±0.11* | 2.93±0.43* |

Note: * - the differences are significant in comparison with the previous period, p≤0.05.

On the 90th day of pregnancy, the area of lymphocyte nuclei in sheep of Stavropol breed was 68.70±2.15 µm², which is 16.72 % (p≤0.05) less than in non-pregnant animals.

By 135th day of pregnancy there was a significant (p≤0.05) increase in the area of lymphocyte nucleus by 14.65%.

In sheep immediately after birth, the area of the nucleus in lymphocytes did not differ significantly from the data of the previous study period.

The study of the number of nucleolar organizer zones in the lymphocyte nucleus revealed differences between the data of the studied age periods of postnatal ontogenesis of sheep (table – 1).

In newborn animals the number of zones of nucleolar organizers in the nucleus of lymphocytes made up 2.90±0.16 units.

By three months of life, this figure significantly decreased by almost 1.8 times.

By the age of six months in experimental animals the number of AgNORs in the lymphocyte nucleus increased by 2.5 times compared to the age of three months.

In nine-month-old sheep, the values of this parameter decreased by 2 times compared to the previous period of the study.

The number of AgNORs in the nucleus of lymphocytes significantly decreased by 40.0%.

In pregnant sheep (table – 2) on 90 days the number of AgNORs significantly decreased by 26.31% compared to non-pregnant animals.
On the 135 day of pregnancy of sheep, the values of this indicator did not change significantly.

In sheep after childbirth, the number of AgNORs increased significantly (by 38.46%, p≤0.05) compared to pregnant females at 135 days and amounted to 2.60±0.11 units.

The average total area of AgNORs in the lymphocyte nucleus of newborn animals (table – 1) was 1.81±0.31 µm² and did not change significantly until the age of three months (1.98±0.23 µm²).

In six months of life there is a significant (p≤0.05) increase in the average total area of nucleolar organizer zones by 2.36 times.

At the age of nine months there is a significant (p≤0.05) decrease in the average total area of nucleolar organizer zones by 1.89 times, and by the age of twelve months, sheep have even lower values of this indicator – 2.56 times lower than in nine-month sheep.

In pregnant sheep (table – 2) compared with non-pregnant animals, the average total area of AgNORs in lymphocyte nucleus did not change significantly up to 135 days.

Afterbirth, the average total area of AgNORs increased significantly (3.76 times, p≤0.05) compared to the data for 135th day of pregnancy and amounted to 2.93±0.43 µm².

4. Conclusions

On the basis of studies of the parameters of the nucleus and parameters of the activity of nucleolar organizers (AgNORs), changes in the morphofunctional state of protein-synthesizing apparatus of blood lymphocytes in sheep of Stavropol breed in postnatal ontogenesis were evaluated. In postnatal ontogenesis and during pregnancy in sheep of Stavropol breed, functional state of lymphocytes changes, this is manifested in the change of parameters of the area of its nucleus, the number and average total area of nucleolar organizer regions. The obtained results can be used as additional criteria in assessing the morphofunctional status of sheep of Stavropol breed and during pregnancy. These data allow us to indirectly judge the level of nonspecific immunity in the body of the sheep of Stavropol breed.

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