Morphological and Functional Activity Dynamics of Blood Lymphocytes in Large White Breed Pigs in Postnatal Ontogenesis and during Pregnancy

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Abstract. The purpose of the study was to research the activity of nucleolar organizers of blood lymphocytes in large white breed pigs in postnatal ontogenesis and during pregnancy. The prepared blood smears were stained with silver nitrate (AgNO₃) and additionally stained with azur and eosin. By measuring the parameters of the nucleus and nucleolar organizers’ activity (AgNORs), the changes in the morphological and functional state of the protein synthesizing apparatus of blood lymphocytes in large white breed pigs in postnatal ontogenesis were evaluated. It was found that the area of lymphocyte nuclei in newborn piglets was 83.72±3.37 mm². By the age of three months, the area of the lymphocyte nuclei of pigs significantly increased by 13.41% (p≤0.05) and amounted to 96.69±2.57 mm², at the age of six months this indicator was 15.78% more than at the age of three months (114.80±3.69), by the age of nine months the number of AgNORs significantly (p≤0.05) decreased by 11.32% compared to six-month-old animals (101.80±2.97). From 1 to 4 units of AgNORs are detected in the lymphocyte nuclei. By the age of three months, the total area of nucleolar organizers’ zones decreased by 29.30% (p≤0.05) and by the age of twelve months increased by 16.72% (p≤0.05) compared to the previous age period. A slight increase in the area of the lymphocyte nucleus was observed in pigs after delivery by 9.15% (p≤0.05). After delivery, the number of AgNORs in pigs significantly increased by 11.26% (p≤0.05). During pregnancy, from 1 to 3 AgNORs were detected in pigs with a total area of 4.29±0.27 to 4.33±0.30 microns. Based on the conducted research, it can be concluded that the functional state of lymphocytes undergoes obvious changes in postnatal ontogenesis and during pregnancy, this can be seen in alterations in the area of their nuclei, the number and total area of AgNORs.

1. Introduction

The study of nucleolar organizer zones' activity (AgNORs) is one of the most relevant methods for determining the morphological and functional state of blood cells.

The nucleolus is an organelle of a cell and its variable structure can be used to evaluate the processes taking place in an animal cell aimed at protein synthesis, since they are functionally related to ribosomes [1]. Acidic non-histone nucleolar proteins (C23, B23, UBF and RNA polymerase) correspond to the transcription processes of ribosomal genes located in the nucleating regions (NPS), regulate the...
proliferative activity of cells and are permanent components of cell nuclei during their functioning [2, 3]. To visualize these mechanisms, the silvering method is used, and by analyzing the quantitative parameters of argyrophilic structures, it is possible to assess the activity of genes in ribosomes. A. F. Lazarev and co-authors [4] indicate that the content of C23 and B23 proteins in the nucleoli directly (by 70%) affects the intensity of staining when identifying zones of nucleolar organizers.

Many domestic and international scientists claim that white blood cells, along with protecting the body from foreign antigens, regulate morphogenesis to maintain normal growth and development of body tissues [5, 6, 7].

Due to the presence of the nucleus, lymphocytes are the most complex functionally active mammalian blood cells, and they also provide a number of vital processes in the body.

Lymphocytes receive signals about any changes in homeostasis and alter their functional activity to restore biological constants. This principle of functioning determines the nature of the reaction of immunocompetent cells, due to which they are indicators of the state of the body as a whole [8].

An increase in the area of the cell nucleus provokes an increase in the activity of AgNORs and the level of expression of various genes [9].

Nucleolus proteins (nucleolin, nucleophosmin, UBF, and RNA polymerase I) are visualized as dark dots (granules) when stained. In these areas, silver is associated with acidic proteins, which are directly related to transcription and transformation rRNA [10].

P. M. Klenovitsky et al [11] exploring the intact lymphocytes of various animals showed that for each species the number of them is specific and closely related to the number of clusters of ribosomal genes detected by silvering.

During light microscopy after silver staining, argentophilic proteins are detected in the interphase only in the nucleoli, and during mitosis – in the region of the nucleolar organizer of mitotic chromosomes and in the perichromosomal material [12].

The above stated leads to the conclusion that the study and detailed description of nucleolar organizers' zones make it possible to assess the proliferative potential and protein-synthetic function of cells.

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

In this regard, the purpose of this research was to study the activity parameters of lymphocyte nucleolar organizers' zones in the blood of large white breed pigs in postnatal ontogenesis and during pregnancy.

2. Materials and methods

During the period from 2016 to 2021, the study of the functional activity of lymphocytes in the blood of large white breed pigs was carried out at the agricultural breeding farm "Russia" in Novoaleksandrovsky District of Stavropol Territory, as well as at the Department of Physiology, Surgery and Obstetrics of Stavropol State Agrarian University.

To achieve this goal, blood samples were taken from clinically healthy large white breed pigs at the age of 1 day (newborns), as well as at the age of three, six, nine and twelve months, and also from non-pregnant pigs, from those that were in the first and second half of pregnancy and after delivery. There were 10 female animals in each experimental group. The feeding ration corresponded to the nutritional standards of the VIZ–VNIIOK (Federal Research Centre for Animal Husbandry).

Blood from pigs for the preparation of smears was obtained by puncture of the ear vein in the morning, before feeding, in test tubes with an anticoagulant (S-Monovette®, SARSTEDT, Germany).

To identify zones of nucleolar organizers in blood lymphocytes, the methods by W. Howell and D. Black (1980), modified by V. I. Trukhachev and co-authors (2015) were used [13].

The study protocol included the following stages:

– preparation of smears from the blood of pigs;
– fixing the material with methyl spiritus and drying;
– rinsing with distilled water;
– immersion in a solution of potassium chloride (0.57 g in 100 ml of distilled water) for 20 minutes;
– rinsing with distilled water;
– immersion and incubation in the dark in a thermostat (370C) in a mixture of equal parts of silver nitrate (50% solution) and 2% gelatin solution (on 1% formic acid solution) for 20 minutes;
– rinsing with distilled water;
– immersion and incubation in the dark in a thermostat (370C) in a 5% Na₂SO₄ solution twice for 8 minutes;
– rinsing with tap water, and then with distilled water;
– finishing of smears by the Romanovsky method;
– rinsing with tap water and drying in a thermostat (370C);
– incarceration in Canadian balsam with cover glasses.

Microscopy of the finished preparations was performed using an OLYMPUS-BX 43 microscope (Japan), and digital images were obtained using an OLYMPUS C 300 camera (Japan). In each preparation, 10 micrographs were performed using ×40 and ×100 fold magnification. The obtained digital photos in 10 lymphocytes were used to measure the area of nuclei, the number and area of nucleolar organizers’ zones (AgNORs), using the software Video Test Morphology 5.1 for Windows (JSC “ISTA”, St. Petersburg).

The data obtained by morphometry were processed using a single-factor analysis of variance and a two-way Student's criterion using the Primer of Biostatics 4.03 program for Windows. The differences were considered significant at p<0.05.

3. Research results

The results of studying the morphometric characteristics of lymphocytes and their zones of nucleolar organizers (AgNORs) in pigs show that the area of the lymphocyte nucleus at three months after birth (figure 1) was 13.41% (p≤0.05) larger than in newborn piglets (figure 2). These data are presented in table 1.

![Figure 1. A lymphocyte with an argentophilic nucleolar organizer. Age - three months. Staining with nitric acid silver with additional staining by the Romanovsky method. Magnification ×400.](image1)

![Figure 2. A lymphocyte with an argentophilic nucleolar organizer. Age - newborn. Staining with nitric acid silver with additional staining by the Romanovsky method. Magnification ×400.](image2)

At the age of six months, this indicator was 15.78% (p < 0.05) more than at the age of three months old. In nine-month-old animals, the area of the lymphocyte nucleus was 11.32% (p≤0.05) less than at the age of six months.

From 1 to 4 units of AgNORs are found in the nuclei of lymphocytes. By the age of nine months, the number of AgNORs significantly (p≤0.05) increased by 16.67% compared to six-month-old animals.
The total area of the zones of nucleolar organizers by three months of life decreased by 29.30% (p≤0.05), and by the age of twelve months increased by 16.72% (p≤0.05) compared to the parameters of nine-month-old animals.

| № item | Indicator                     | Age                  |
|--------|-------------------------------|----------------------|
|        | Newborn (n=10)               | Three months (n=10)  |
|        | 83.72±3.37                   | 96.69±2.57*          |
| 1      | The area of the lymphocyte nucleus, mm² | 114.80±3.69*           |
|        |                               | 101.80±2.97*         |
|        |                               | 102.65±3.23          |
| 2      | Number of AgNORs, item       |                      |
|        | 1.42±0.29                    | 1.38±0.06            |
|        | Total area of AgNORs, mm²    | 1.35±0.08            |
|        | 6.04±0.18                    | 4.27±0.32*           |
|        |                               | 5.43±0.56            |
|        |                               | 5.08±0.64            |
|        |                               | 6.10±0.12*           |

* - the differences are relevant with the previous period of the study, p≤0.05.

A slight increase in the area of the lymphocyte nucleus by 9.15% was observed in pigs after delivery (table 2).

During pregnancy, from 1 to 3 AgNORs were detected in pigs. After delivery, the number of AgNORs in pigs significantly increased by 11.26%. The average values of the total area of nucleolar organizers' zones in the nuclei of lymphocytes did not undergo significant changes during pregnancy. Their maximum was observed in the first half of pregnancy.

| № item | Period of pregnancy       | The area of the lymphocyte nucleus, mm² (M±m) | Number of AgNORs, item (M±m) | Total area of AgNORs, mm² (M±m) |
|--------|----------------------------|---------------------------------------------|-------------------------------|---------------------------------|
| 1      | Not pregnant (n=10)       | 90.85±2.17                                 | 1.22±0.05                     | 3.92±0.39                       |
|        | First half of pregnancy   | 90.34±1.94                                 | 1.35±0.55                     | 4.33±0.30                       |
| 2      | (n=10)                    |                                            |                               |                                 |
| 3      | Second half of pregnancy  | 92.76±2.37                                 | 1.34±0.08                     | 4.29±0.27                       |
|        | (n=10)                    |                                            |                               |                                 |
| 4      | After delivery (n=10)     | 102.10±2.21*                               | 1.51±0.08*                    | 3.92±0.31                       |

* - the differences are relevant with the previous period of the study, p≤0.05.

Thus, the research conducted to determine the structural organization and phases of blood lymphocytes' activity showed that in pigs after birth, the area of the lymphocyte nucleus varies from 83.72±3.37 to 102.65±3.23 mm², the number of zones of nucleolar organizers varies from 1.42±0.29 to 1.54±0.09, with a total area of AgNORs from 6.04±0.18 to 6.10±0.12 mm², while with age, changes in these parameters have a wave-like character, and after six months of life reach maximum values. During pregnancy, from 1 to 3 AgNORs were detected in pigs, their total area varies from 4.29±0.27 to 4.33±0.30 microns. The obtained digital data on the number of AgNORs are consistent with the results of studies obtained by L. K. Ernst and co-authors who studied them in pigs [14].

The highest values of lymphocytes and AgNORs from the age of six months, in our opinion, are related to an increase in the functional activity of these cells during puberty. The results obtained during the study are consistent with the data of L. A. Grebnyak (2003), who argued that the greatest plasticity
of the genome and the maximum variability of the nuclear parameters of cells are detected during puberty.

4. Conclusion
Based on the studies of the parameters of the nucleus and nucleolar organizers’ activity (AgNORs), the changes in the morphological and functional state of the protein synthesizing apparatus of blood lymphocytes in large white breed pigs in postnatal ontogenesis were evaluated. In postnatal ontogenesis and during pregnancy, the functional state of lymphocytes in large white breed pigs undergoes significant changes, which can be observed in the parameter changes of their nuclei area, the number and average total area of the regions of the nucleolar organizers. The obtained results can be used as additional criteria for assessing the morphofunctional status of large white breed pigs in postnatal ontogenesis and during pregnancy. These data make it possible to indirectly evaluate the level of nonspecific immunity of the pig’s body.

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