Immunobiological mechanisms of stimulation of the body's natural resistance in conditions of altered reactivity

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Abstract. The paper studied the effect of microbial polysaccharides on the leukogram of laboratory animals and studied the dynamics of peripheral blood parameters with an assessment of immunobiological reactivity based on established patterns of physiological status. The results of numerous studies of the state of natural resistance of farm animals indicate that protective forces are a dynamic indicator, and is determined both by the genetic characteristics of the body and the impact of various environmental factors. This circumstance allows directionally influencing the formation and manifestation of the body's defenses. Thus, the study of the immunological reactivity of the organism of farm animals becomes relevant for understanding the pathogenesis of diseases, for rational pathogenetic therapy. The problem of increasing the nonspecific resistance of productive animals has not lost its relevance in the current difficult economic situation in Russia. This fact is dangerous by the manifestation of enzootic outbreaks of infectious diseases and a gradual decrease in the breeding value of animals.

1. Introduction

One of the most important tasks of applied immunology is the development of ways and methods for increasing the immunobiological status of newborn animals, aimed at more complete formation of the humoral and cellular link. The most promising direction is immunocorrection in newborn animals, by intramuscular administration of highly active non-specific immunomodulators of a wide spectrum of action. Currently, the growth of immunodependent pathology, i.e. diseases based on immunopathological mechanisms, is increased. Among them are violations of anti-infection protection, proliferation and regeneration, allergic and autoimmune processes. A feature of the course of diseases with immunopathological conditions is, as you know, early chronicity, torpidity to traditional methods of therapy and the need for immunorehabilitation [3].

The problem, despite its extreme relevance and great practical significance, the growing interest of a wide circle of researchers still, does not have clear methodological approaches.

With the current ways and methods of animal breeding, the study and identification of patterns of formation of immunobiological status is a promising direction and requires a careful approach and resolution. The process of formation of the immunobiological system itself acts as a complex of factors determining it for newborn animals.
Studies on the formation of the immunobiological status of animals were carried out by a number of domestic scientists. However, many questions on the formation of the immunobiological potential in the early neonatal period and the factors affecting its formation have not been studied enough.

Scientists have established the dependence of the onset and spread of diseases with a decrease in the immunobiological potential and specific reactivity in newborns [4].

The priority and most significant period for the newborn organism is the moment of birth and the neonatal period. To realize the adaptive potential of the newborn organism at these stages of postnatal ontogenesis, full-fledged environmental conditions are necessary. Important problems during these periods are temperature and immunobiological stresses. This is due to the entry of the newborn organism into a different habitat with a lower temperature and with a reduced perfection of the immune response.

With the current intensification of animal breeding, in rapidly changing technological conditions, the newborn organism does not have time to timely switch to a new level of functional activity, and there arises a strain of homeostatic characteristics, a decrease in the immunobiological potential and the underdevelopment of specific defense mechanisms.

The physiological immaturity of the immune system is inherent in the newborn organism, which is compensated by the secretion of maternal colostrum. The immune system maintains the structural and functional integrity of the body and potential for recovery are the structural link of the immunobiological status, and the establishment of a functional profile after birth is of particular interest to neonatology and immunology in general. Modern views of the dependence of the formation of a functional state and the improvement of the immunobiological system require a more extended and in-depth study of this issue [1].

It was believed that the immune system was provided with the main function of maintaining antigenic constancy, but, despite this, it is involved in the formation and restoration of relative constancy in the internal environment of the body under various physiological conditions.

To counter many etiological pathogenic substances, newborn animals must have a wide functional maturity potential, which can be realized at a high level of immunobiological status.

The formation of the immunobiological system requires the mobilization of the metabolic potential, therefore, the stability and viability of the organism depend on the possibility of the formation of functional features.

Nonspecific resistance of the body includes a number of tissue and humoral reactions that determine the degree of resistance to various damaging influences. The intensity of these reactions depends on numerous endogenous and exogenous factors. These factors include, in particular, the functional state of the nervous system, endocrine regulation, good nutrition, seasonal and climatic factors [3].

Veterinary medicine, which did not have specific pharmacological preparations, was for a long time limited by means and methods that enhance the body's natural resistance, and in some cases tangible results were achieved. This applies, in particular, to the treatment of various infections [6].

As rightly noted by a number of authors (3.V. Ermoleva, I.A. Kassirsky, G.P. Rudnev, A.A. Bogomolets, A.F. Bilibin, and others), the success of treatment largely depends on the intensity protective and reparative reactions of the patient. Without a sufficiently high level of these reactions, the effectiveness of various specific therapeutic agents is insufficient to ensure successful treatment of the patient [2].

Currently, the causes and mechanisms of antibiotic resistance are well understood. It has been established that, in addition to the variability of microorganisms, their high adaptive ability, the genetic transfer of resistance as a hereditary trait is also involved in the formation of stable variants of microorganisms. Special extrachromosomal transmission factors of this feature, the so-called R-factors, were also identified. However, identifying the causes and mechanisms of the emergence of resistance of microorganisms contributed little to finding reliable methods for its prevention and suppression of resistant strains [10].

At present, there is no doubt that the immune system is one of the first to respond to the adverse effects of the external environment and is the most important component in the set of compensatory-adaptive mechanisms that determine the adaptation of the organism as a whole. Dysfunction contributes
to the emergence of dysadapative states, which are the basis for the development of various diseases [7].

A high level of functional reserves of the pregnant body is important in the prevention of intrauterine infection. Infection in the prenatal period of development affects the processes of growth and development of the fetus on one hand, and on the other hand, the maternal organism is iso-immunized with antigens of the fetus, accompanied by an increase in the sensitivity of the body with a predominant manifestation of cellular phenomena, in the absence of enhanced synthesis of antibodies. Given the high importance of the functional reserves of the newborn, intrauterine development and the usefulness of the placental barrier are important [8].

The above limitations urgently require the introduction of additional treatment methods that contribute to the success of therapy. From this point of view, the development of methods to increase the body's natural resistance is of great importance. An example is the reduction in resistance to agammaglobulinemia, leucosis, myeloma, radiation injuries, protracted infections (tuberculosis), and malignant neoplasms [5].

In recent years, it has been established that some bacterial polysaccharides are distinguished by a pronounced ability to influence the body's immunobiological reactivity. They cause a multicomponent protective reaction of the body, causing a change in the level of resistance [9].

2. Materials and research methods

Research experiments were performed in the conditions of the educational and scientific vivarium of the Faculty of Veterinary Medicine of the Federal State Budget Educational Establishment of Higher Education in the Stavropol State Agrarian University.

The effect of microbial polysaccharides on the leukogram of laboratory animals was studied. The introduction was carried out parenterally (intramuscularly). The test medication "Pyrogenal" was used. The content of hematological indicators was determined on the Automated Veterinary Hematology Analyzer PCE – 90 VET device. Hematological indicators were determined on a ChemwellCombi V 1.03 automated biochemical and enzyme immunoassay analyzer (USA).

The functional activity of neutrophils was evaluated by phagocytic activity (FAN%), phagocytic index (PI), phagocytic blood capacity (PBC) - according to D.K. Novikov (2001). Bactericidal activity of blood serum - according to O.V. Smirnova and T.A. Kuzmina (1966), and lysozyme activity of blood serum - according to V.T. Dorofeychuk (1998).

Digital data were processed by biometric methods according to N.A. Plokhinsky (1987), using the applied computer programs MicrosoftExcel and BioStat.

The present work is based on clinical observations and results of newborn screening. A clinical assessment of the health status of newborns included an assessment of neonatal adaptation, an analysis of the incidence, laboratory screening data, taking into account the data of standard registration forms. The assessment of the state of health provided the analysis of the obstetric and gynecological history, the course of the pregnancy and parturition, laboratory screening data and was carried out using data from standard accounting forms.

The main hematological parameters were determined, and a morphological study of peripheral blood was performed using light microscopy by immunofluorescence using monoclonal antibodies to surface cell differentiation clusters (CD-ClusterDesignation) of antigens (Quantitative determination of blood lymphocyte subpopulations using monoclonal antibodies).

The method of phenotyping the subpopulations of umbilical cord blood and peripheral blood lymphocytes by isolating them on a density gradient with subsequent staining of cells with fluorescently-labeled antibodies and counting the number of cells studied the immune status of newborns, the population composition of blood lymphocytes.

The technical result that has been achieved is reduced to the prophylaxis of the immunodeficiency state of newborns in the fetal period by implementing an immunomodulating effect in the mother's body during the most stressful period (second half) of pregnancy.
This technical result was tested on control in relation to the experimental groups and the offspring received from them. It is carried out due to the intramuscular administration of a highly active non-specific immunomodulator of a wide spectrum of action - Pyrogenal - to pregnant animals at a dose of 0.08 μg / kg of the body weight.

The essence of the method consists and is due to its ability to activate macrophages, enhance phagocytosis (leading to an increase in antimicrobial resistance and accelerated antibody production) and the production of endogenous interferon, stimulate the production of interleukin-1 (IL-1), which causes proliferation of a number of body cells (fibroblasts, endothelial cells, hematopoietic cells, etc.), interleukin -2 (IL-2), necessary for the growth of lymphocytes (primarily of T-cells). Thus, macrophage-monocytic cells and the cytokines secreted by them are activated, the functional activity of both the cellular and humoral units in the mother-fetus functional system is enhanced.

It should be noted that the mother's body, in critical periods of pregnancy, is in a state of physiological immunodeficiency associated with intensive formation and development of the fetus, preparation of the body for the production of colostrum and milk.

At the same time, due to the principle of interconnectedness and interdependence of homologous functional systems in the mother-fetus-newborn complex, it is possible to activate the own biological reactions of the newborn by acting on the mother’s body during gestation, in order to move to a better level of functioning immediately after birth.

It was found that newborns in the earlier stages of morpho-functional development in comparison with other farm animals, are in a state of physiological immunodeficiency, which necessitates the use of immunostimulatory preparations in the fetal period of development, thereby enabling to obtain and produce more full-fledged breed.

3. Results
In the animal experiment, the bacterial polysaccharide Pyrogenal was used and the biphasic nature of the reaction was invariably noted: in the first hours after the administration of LPS, some leukopenia occurred, which was replaced after 2–3 hours with leukocytosis with relative granulocytosis. Parenteral administration of microbial lipopolysaccharides (LPS) to an animal causes characteristic changes in the leukogram.

It was found that at a dose of 20 mg / kg administered parenterally to white mice, the preparation causes a two-phase change in the number of leukocytes in the peripheral blood. 24 hours after administration, the number of leukocytes decreased to 20–50% of the initial value. The maximum level is noted after different periods (from 1 to 4 days). After the Pyrogenal administration of 5–25 mg / kg, leukocytosis with neutrophilia occurred in rabbits after a leukopenic phase lasting 2–6 hours. Leukocytosis and increased phagocytosis lasted up to 14 days.

The individual minimum immunomodulating pyrogenic dose was determined by intramuscular injection of the preparation at the rate of 0.16, 0.08, 0.04 μg per 1 kg of body weight. The most optimal dosage for animals was 0.08 μg per 1 kg of body weight, which contributed to an increase in total body temperature up to 0.5 °C, which indicates the sufficiency of the optimal pyrogenic dose. 1 hour before the injection, each pregnant female was taken temperature measurements twice with an interval of at least 30 minutes. Differences in temperature readings in the same animal did not exceed 0.1 °C. The average temperature increase was up to 0.5 °C.

To establish the positive effect of the Pyrogenal use, 3 groups of 10 animals were formed - two experimental and one control and the breed born from them of 10 animals each. The first of which was injected intramuscularly with 0.08 μg per 1 kg of body weight of the preparation 60 days before the parturition, once a day until morning feeding with a total course of 15 injections. The second experimental group at a dose of 0.08 μg per 1 kg of body weight for 60 days before the parturition every day (5 injections), 40 days before the parturition with an interval of 1 day (5 injections), 20 days before the parturition with an interval of 2 days (5 injections), a total course of 15 injections. The third group served as a control, which did not use the above preparation. The duration of use was 30 days.
It was found that during the injection of Pyrogenal in experimental animals from the second half of pregnancy, the indices of natural resistance significantly increased, with normalization of the parameters of the cellular and humoral immunity, eliminating the signs of an immunodeficiency observed from the second half of pregnancy. However, the use of the proposed dosage and the frequency of administration of the preparation for the experimental groups found their differences in the studied parameters.

In the experiment, it was determined that under the influence of bacterial LPS, bone marrow granulocytes are mobilized, which he calls reserve. In an animal experiment, this mobilization is manifested by an increase in the number of peripheral blood granulocytes after 3-4 hours after the LPS administration. Under the influence of certain microbial polysaccharides in the bone marrow, the maturation of young cells and their release into the blood are accelerated. A single parenteral administration of Pyrogenal causes the same changes in the number of leukocytes (Table 1).

The data in Table 1 indicate an enhanced release of leukocytes into the peripheral blood. A study conducted on experimental groups of animals showed that depending on the administered dose of Pyrogenal, the number of granulocytes in peripheral blood increased at different times.

**Table 1.** The effect of "Pyrogenal" on the number of leukocytes thousand / μl of blood of experimental animals.

| Time after injection, h | Mice  | Guinea pigs | Rabbits |
|------------------------|-------|-------------|---------|
| Before injection       | 8.4   | 11.0        | 9.5     |
| 3                      | 5.8   | 4.2         | 6.3     |
| 6                      | 16.3  | 17.5        | 15.2    |
| 24                     | 14.9  | 11.3        | 8.7     |
| 48                     | -     | 12.1        | 9.3     |
| 72                     | 10.3  | 14.2        | 9.3     |

At a dose of 0.8 μg / kg, this reaction occurred within 5–6 hours, and at higher doses, 3-4 hours after administration of the preparation. Along with this, the cellular composition of the leukogram changed (Table 2).

**Table 2.** The effect of “Pyrogenal” on the number of neutrophils (in percent) in the blood of animals.

| Time after injection, h | Mice  | Guinea pigs | Rabbits |
|------------------------|-------|-------------|---------|
| Before injection       | 36    | 27          | 39      |
| 3                      | 59    | 51          | 18      |
| 6                      | 53    | –           | –       |
| 24                     | 49    | 47          | 60      |
| 48                     | 40    | 35          | 61      |
| 72                     | 36    | 39          | 44      |
| 96                     | –     | 35          | –       |

The smallest values, according to the data, were noted the day after the injection. Then, after 3 days, the number of leukocytes increased significantly (up to 300% and higher).

After intramuscular administration of Pyrogenal to rats, leukopenia most often occurred in the first 2 hours, followed by leukocytosis. The number of leukocytes in 1 μl of blood reached 30–40% of the initial value. The maximum increase was observed in most animals 6–8 hours after injection. Basically, the neutrophil content increased (from 30 to 45%), which was accompanied by an increase in the number of immature stab forms. The change in the level of leukocytes was less pronounced with repeated administration of the preparation.

In all cases, after the injection, followed by short-term leukopenia (2–4 hours), leukocytosis with neutrophilia developed. In some cases, neutrophilia was accompanied by relative eosinophilia. After each injection, a uniform reaction occurred, in which after 2 hours there was a certain decrease in the
number of leukocytes circulating in the blood, and after 4 hours their significant increase (2 times or more). A day later, the initial level was restored. After the 3rd and 4th injection, the numbers were slightly lower than after the 1st and 2nd, which indicates a slight decrease in the intensity of the reaction.

It was found that on the 10th day after birth, the indicators of immunobiological potential were at a fairly high level. This was expressed in the fact that in the quantitative composition of T- and B-lymphocytes this group exceeded the control individuals reliably in T-lymphocytes by 51.1% and by 53.9% in B-lymphocytes. This condition can be characterized as a pronounced process of leukopoiesis in the second group.

Indicators of phagocytic activity of neutrophils (FAN), bactericidal (BABS) and lysozyme (LABS) activity of blood serum in the breed indicate that the level of non-specific humoral protection in animals from the experimental groups was more expressed. So FAN for the first group was at the level of 37.25%, BABS - 31.47%, and LABS - 34.21%, for the second FAN - 41.62%, BABS - 39.30% and LABS - 43.46%. However, in the control group, these values were FAN - 31.74%, BABS - 27.61% and LABS - 30.08%.

After the injection, the number of leukocytes began to increase and reached a maximum over 3 days. White blood cells were represented mainly by neutrophils. In a number of cases, a left shift was noted (stab cells, less often young cells). The use of LPS caused the greatest increase in both the absolute and relative number of neutrophils, that is, cells actively participating in phagocytosis. Increased phagocytosis occurs not only due to quantitative changes in the composition of cells, but to an even greater extent due to qualitative changes occurring in leukocytes. These shifts are characterized by an increase in adhesive ability, activity of intracellular enzymes, and digestion activity.

4. Conclusions
Under the influence of Pyrogenal, a complex reaction of activation of a whole complex of protective and adaptive reactions develops. There is reason to believe that many of them are still unknown to researchers, since in general a complex phenomenon, denoted by the general terms “immunobiological reactivity” of an organism with a modern level of knowledge, is not completely decrypted.

All of the functions that were studied in the experiment turned out to be highly sensitive. A detailed study of these mechanisms allows more targeted use of such compounds, taking into account the peculiarities of the multicomponent reaction caused by them in the animal’s body.

It should be noted that the mother’s body, in critical periods of pregnancy, is in a state of physiological immunodeficiency associated with intensive formation and development of the fetus, preparation of the body for the production of colostrum and milk.

However, due to the principle of correlation and interdependence of homologous functional systems in the complex “fetus-mother-newborn” may activate the newborn's own biological response by affecting the parent organism during gestation, to move to a better level of functioning immediately after birth.

The change in the formation of the main immunobiological and morpho-functional indicators in the neonatal period gives reason to assert the most plastic adaptation of newborns from the experimental group in relation to the analyzed experimental and control.

These changes contribute to the creation of a higher level of adaptive, metabolic potential and immunobiological status in the born breed.

The above data, the use of the proposed method of immunobiological correction in newborn animals can serve as the basis for the prevention of postnatal complications.

Based on comprehensive clinical and laboratory studies, the state of the cellular component of immunity at birth was found for the first time. The studies performed allowed us to approach the assessment of the cellular composition of immunity at birth at a methodologically new level and to reveal its significant decrease in comparison with similar indicators.

The clinical and immune response of newborns at birth to the use of various corrective therapy methods has been determined, which allows us to give a scientific justification for pathogenetic therapy.
It has been established that newborn animals are born at earlier stages of morphofunctional development, in a state of physiological immunodeficiency, which necessitates the use of immunostimulating preparations precisely in the fetal period of development, thereby allowing more complete breed to be obtained and raised. The change in the formation of the main immunobiological and morphofunctional indicators in the neonatal period gives reason to assert the most plastic adaptation of newborns from the 2nd experimental group in relation to the analyzed experimental and control ones.

The mismatch between the level of adaptive-compensatory capabilities and functional loads on the pregnant body is the main predisposing link in the concomitant pathology.

One of the main causes of neonatal diseases in a newborn is a transferred immunosuppressive state in the fetal period of development.

Under conditions of altered homeostasis, the mother’s body decreases the transport of oxygen and nutrients to the fetus. It was found that normal birth depends on the state of regulatory mechanisms during pregnancy. The formation and development of the placenta is disrupted. This leads to the biochemical, enzymatic and morphological changes in a unified system “mother-placenta-fetus”.

The immediate result of the immunobiological effect is the development of placental insufficiency in pregnant individuals, which reduces the adaptive capacity of the breed and can cause its death in the early peri- and postnatal periods.

The most important mechanism for full adaptation to environmental conditions is immunobiological reactivity. The condition of the pregnant is one of the main reasons for the decrease in the natural, cellular and humoral links of immunity in the born breed.

According to modern research, an immunoreactive state accompanies any pathology. This is what determined the scientific interest in the problem of correcting this pathogenetic effect in a pregnant body.

From the conducted studies, it can be concluded that the immunoregulatory state of the mother’s body leads to significant changes in the immunobiological status of the breed received from them.

The level of physical development of newborns, depending on the presence of signs of immunosuppression, significantly differed in all assessed indicators (metabolic rate).

Thus, it was found that the quality of the breed received from mothers with signs of immunosuppression in the second half of pregnancy indicates a direct or indirect effect of this pathogenetic effect on the fetoplacental complex.

This implies the importance of the problem of the prevention and correction of this negative effect in the biological complex "mother-fetus-newborn". This condition is a necessary link for maintaining high productivity, fertility and obtaining a viable breed.

The ongoing correction of the immune state of the mother’s body contributes to the creation of a higher level of adaptive, metabolic and immunobiological potential in the breed born. This was expressed by reliable changes in relative and absolute indicators of the formation of immunobiological status, taking into account their age.

The above data, the use of the proposed method of immunobiological correction in newborn animals can serve as the basis for the prevention of postnatal complications.

These changes contribute to the creation of a higher level of adaptive, metabolic potential and immunobiological status in the born breed.

References
[1] Milovanovic M, Dietze K, Milicevic V, Radojicic S, Valiec M, Moritz T, Hoffmann B 2019 BMC Vet Res. 15 pp 56-61 doi: 10.1186/s12917-019-1831-y
[2] Brunse A, Worsoe P, Pors S, Skovgaard K, Sangild P 2019 Shock 51 pp 337-347 doi: 10.1097/SHK.0000000000001131
[3] Dennis M, Eudailey J, Pollara J, McMillan A, Cronin K, Saha P 2018 J Virol. 93 pp 64-78 doi: 10.1128/JVI.01783-18
[4] Zheng T, Crews J, McGill J, Dhume K 2018 Parasite Immunology 41 pp 228-238 doi: 10.1021/acsinfectdis.8b00213
[5] Iraola G, Perez R, Betancor L, Marandino A, Morsella C, Mendez A 2016 BMC Veterinary
Research 12 pp 103-111 doi: 10.1186/s12917-016-0913-3

[6] Seguel M, Perez-Venegas D, Gutierrez J 2019 Physiological and Biochemical Zoology 92 pp 326-338 doi: 10.1086/702960

[7] Karussis D, Petrou P 2018 Immunologic Research 92 pp 642-648 doi: 10.1007/s12026-018-9032-5

[8] Dai J, Yang X, Zhu Y, Wang C 2018 Cell Therapy Against Cerebral Stroke 50 pp 3797-3803 doi: 10.1016/j.transproceed.2018.05.019

[9] Karussis D, Petrou P 2018 Immunologic Research 7 pp 368-372 doi: 10.1007/s12026-018-9032-5

[10] Alvarez-Rodriguez M, Atikuzzaman M 2019 International Journal of Molecular Sciences 20 pp 502-522 doi: 10.3390/ijms20030513