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Development of a method for estimation functional reserves of a newborn organism.

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ABSTRACT

Given the high importance of the functional reserves of a newborn organism, intrauterine development and the usefulness of the placental barrier are important. The barrier function of the placenta is manifested only in physiological conditions. Under the influence of pathogenic factors, the barrier function of the placenta is disrupted, and it becomes permeable even for such substances, which under normal physiological conditions pass through it in a limited amount. Violation of the placental barrier affects the growth and development of the fetus on the one hand, and on the other hand, the maternal organism is isoimmunized with fetal antigens, accompanied by an increase in the sensitivity of the organism with the predominant manifestation of cellular phenomena, in the absence of enhanced antibody synthesis. The state of hypersensitivity of the maternal organism to fetal antigens is established in the reaction of leukocytolysis of cells in the presence of specific proteins of the newborn organism.

Keywords: immuno-dependent pathology, immunopathological mechanisms, immune rehabilitation, reactivity, sensitivity, pregnant animals

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INTRODUCTION

The aim of the proposed invention was to develop a method for evaluating the functional reserves of a newborn organism, aimed at a more relevant evaluation of the functional reserves of newborn animals, by a biological test based on the reaction of the leukocytolysis of the blood of the mother organism in the presence of the blood serum of a newborn animal that has the acceleration, simplification, and increased accuracy in predicting the development of the newborn.

The technical result was achieved by evaluating the functional reserves of a newborn organism, which included a blood test with a biological test, which was used as the reaction of the leukocytolysis of the blood of the mother organism with the presence of the serum of the newborn. All this amounted to determining the viability of a newborn animal by performing contrasting blood leukocytolysis reactions in the "mother-newborn" biological system.

This technical result was carried out due to the detection of the state of hypersensitivity of the maternal organism to the fetal antigens by the reaction of leukocytolysis of cells in the presence of specific proteins of the newborn organism. Proteins of blood serum from isoimmunized newborns cause specific damage to sensitized blood cells of the mother's body and do not affect leukocytes, which do not show sensitivity to them. Thanks to this, according to the established formula, the percentage of lysed leukocytes of the maternal animal was calculated under the influence of the serum of the newborn's animal. After that, the reaction was evaluated and the prognosis of the functional reserves of the newborn organism was established.

Currently, more and more attention is paid to the development of biochemical diagnostic methods based on multi-link inter-system processes that reflect pathogenetic, compensatory, adaptive shifts associated with its complicated course. But, despite the variety of methods, they cannot always be used in the diagnostic process due to complexity, and sometimes because of low information content; in addition, many of them do not have the necessary specificity. Therefore, the problem of creating a fairly simple, accessible to a wide range of therapeutic veterinary institutions, and at the same time, a reliable way to predict the development of the newborn remains relevant.

MATERIALS AND METHODS

In two tubes, add 0.3 ml of mildly-colored methylene blue solution of acid-pepsin at a concentration of 25 mg/ml. In the first (experimental) 0.1 ml of blood serum of a newborn is added, and in the second (control) - 0.1 ml of sterile physiological solution. Then, 0.02 ml of blood from the mother animal is added to each tube. The samples were thoroughly mixed and placed in a thermostat for 2 hours at 37.5 ° C.

The acid medium of a solution of acid-pepsin creates conditions for the osmotic destruction of erythrocytes of the blood being studied, the leukocytes remain undisturbed.

Proteins of newborn serum contained in the test sample cause specific damage to the sensitized blood leukocytes of the mother's body and do not affect the white blood cells, which do not show increased sensitivity to them. To detect the damage to white blood cells by counting is possible only if they are completely destroyed. Destruction of damaged leukocytes is carried out by enzyme pepsin, which in acid medium acts only on damaged cells and does not act on undamaged ones. Such selective action of pepsin on damaged leukocytes provides a difference in their calculation.

After incubation, the contents of both tubes are alternately filled in the Goryaev chamber and count the number of white blood cells in 25 large squares according to the generally accepted trivial technique adopted for counting leukocytes.

To calculate the percentage of lysed leukocytes of a maternal animal under the influence of the blood serum of a newborn animal, the formula is used:

$$IAAL = \frac{L_{\text{control}} - L_{\text{test}}}{L_{\text{control}}} \times 100\%$$

IAAL – an indicator of allergic alteration of leukocytes;
L – number of leukocytes.

RESULTS AND DISCUSSION

In the first days after lambing in sheep and their offspring (lambs), blood samples were taken and indicators of allergic alteration of leukocytes were determined. The results are summarized in Table 1.

Table 1: Parameters of leukocyte allergic alteration in the animals under study

№	Individual number of the animal	Number of leukocytes, thous.		The index of leukocyte allergic alteration,%
		Control	Test	
1.	8333	4,0	2,0	50,0
2.	8561	5,8	3,8	34,5
3.	8661	5,8	4,0	31,03
4.	8914	7,8	6,6	15,8
5.	7274	5,2	4,0	23,07
6.	3643	4,0	4,0	–
7.	7746	7,8	7,2	7,69
8.	8430	5,6	5,6	–
9.	8120	5,6	5,6	–
10.	8148	7,6	7,4	2,63

So, 70 ewes were examined in total and 22 of them had increased sensitivity to the serum proteins of newborns, i.e. the indicator of leukocyte allergic alteration was 10% and higher.

Lambs born from ewes with hypersensitivity to fetal antigens (the indicator of leukocyte allergic alteration of 10% and more) were less viable, which is confirmed by next example.

The proposed method was studied in two groups of newborn lambs. The experimental group included lambs, whose queens had increased sensitivity to newborns' antigens. The control group is represented by lambs from non-sensitized ewes. Differences between ewes by the indicators of leukocyte allergic alteration were statistically significant. This indicator in the group of ewes with increased sensitivity was $26.90 \pm 0.59\%$, and in the control group - $6.49 \pm 0.39\%$.

The lambs of both groups at the 24-hour age were examined for blood by the indices of natural resistance. In 7 months, morbidity and mortality of animals were taken into account. The results of the studies are shown in Table 2.

Table 2: Indices of natural resistance in the animals under study

Indicators	Unit. measurements	Groups of lambs (n=50)	
		Test (n=25) M±m	Control (n=25) M±m
1. Living weight	kg	3.796±0,12	4.596±0,19
2. Erythrocytes	1012 (million)	10.41±0,56	13.63±0,28
3. Hemoglobin	g / l	96,3±0,46	114,2±0,22
4. Leukocytes	109 (thousand)	3,49±0,24	3,64±0,29
5. ESR	mm for 24 hours	5,88±0,75	3,44±0,19
6. Phagocyte activity (FAN)	%	68,72±3,02	85,76±1,61
7. Fagotsitarnaya intensity (FI)	mcd.	3,12±0,20	3,79±0,34
8. Fagocyte number (FH)	mcd.	2,26±0,23	3,33±0,35
9. Lysosyme activity (LASK)	%	13,40±1,41	18,40±1,04

As can be seen from Table 2, the experimental and control groups of lambs differed significantly from each other in terms of indices characterizing the state of natural resistance. When analyzing hematological parameters, it was established that lambs born from sensitized ewes have a smaller number of erythrocytes in 1 mm³ of blood (10.41 ± 0.56) than in lambs of the control group (13.63 ± 0.28). Identical differences are observed between groups of animals in terms of hemoglobin content, erythrocyte sedimentation rate (ESR). According to the content of leukocytes in 1 mm³ of blood, there are no significant differences between groups of animals. However, the functional activity of leukocytes in the animals of the experimental group was significantly lower than in the control group.

The phagocytic activity of neutrophils in the lambs of the control group was $85.76 \pm 1.81\%$ at the daily age, and in the lambs of the experimental group, it was $68.72 \pm 3.02\%$, respectively. There were differences in other parameters of leukocyte phagocytosis (FI, FCH).

Lysozyme activity of blood serum also had significant differences, and these parameters in the control group significantly exceeded the experimental one.

The offspring (lambs) of the experimental and control groups differed in their live weight. So the body weight in the animals of the experimental group was 3.796 ± 0.12 kg, and in the control animals - 4.596 ± 0.19 with a statistically significant difference, respectively.

Thus, according to the indices of natural resistance in the diurnal age, lambs born from queens with increased sensitivity to serum proteins differed significantly from their peers. A weak manifestation of natural resistance and a lower weight of lambs in the experimental group are associated with a violation of the placenta barrier, the phenomena of isoimmunization of the maternal organism by fetal antigens and the adverse effect of the immunological factors of the maternal organism on the growth and development of the fetus.

Evidence of the fact that isoimmunization during pregnancy took place may be an increase in the rate of erythrocyte sedimentation and a decrease in their number in the lambs of the experimental group relative to the control group. It is known that isoimmune antibodies, and such sensitized leukocytes, entering the fetus via the placenta or colostrum, enter an immunological reaction with the newborn's red blood cells, which contain antibodies. This leads to a change in the colloidal properties of the erythrocyte membranes, as a result of which they easily agglutinate and undergo destruction.

An important criterion for the viability of newborn animals is the incidence and mortality in the process of ontogeny. As a result of the tests, it was found that at the age of one month the incidence in the experimental group was 16.0%, and in the control 4.0%. Mortality in the experimental group was 12.0%. In the control group, cases of death were not recorded. Further observations of lambs up to 7 months of age showed that the incidence of morbidity and mortality in the experimental group is significantly higher than in the control group. In total, 24.0% fell in the experimental group, and 4.0% in the control group.

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CONCLUSION

It can be concluded from the study that the proposed method has the following advantages:

1. The proposed method is for evaluating the immunobiological status of a newborn.
2. It is less labor intensive in application, making it simple and accessible for technical implementation in any agricultural enterprise.
3. There is no need for invasive and stressful manipulation in newborn animals in the early periods of postnatal development, which increase their sensitivity, with possible complications.

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