The Combined Effect of STEMB and Immunomodulatory Substance in the Blood Profile of Cows at the 15th Day Post-Partum

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The objective is to determine the morphological and biochemical blood parameters of cows at the 15th day postpartum with the separate and combined use of an Embryonic Stimulator (STEMB) and an immunomodulator (Immunopharm). In all studies, there were three experimental groups. For the first experimental group, the STEMB solution was administered to Holstein cows at 30 days before delivery, injected thrice subcutaneously at 7 days' intervals and at a dosage of 0.075 ml/kg of body weight. The second experimental group was administered with the immunomodulator thrice intramuscularly at 7 days' intervals and at a dosage of 6 ml per cow, starting 30 days prior to delivery. Finally, the third experimental group of cows were administered the drugs STEMB and Immunopharm combined while using the same dosage (0.075 ml/kg and 6 ml/cow respectively) and scheme (Thrice, 7 days' interval, starting at 30 days before deliver) as the two other groups. It was found that the combined use of STEMB and Immunopharm improved the blood cell composition; increased amount of monocytes, segmented neutrophils, hemoglobin, total protein, alpha-globulins, immunoglobulins (A, M, G) and also reduced the indicators of ALT and AST enzymes to the threshold level. It has been demonstrated that the combined use of STEMB and Immunopharm are optimal for the prevention of birth and postpartum complications as well as providing an improvement in the blood cell composition.

Keywords: ALT; AST; Embryonic; Immunomodulator; Postpartum.

In modern conditions of farm management, preventing postpartum complications and infertility is one of the major challenges for veterinary science, especially for high-yielding dairy cows. According to recent scientific studies, the main cause of pathologies during pregnancy, childbirth, and postpartum in animals are metabolic disorders which are confirmable by blood tests[1-3].

The parameters of blood as a connective tissue are most thoroughly studied and its morphological and biochemical composition are one of the main sources of information. However, blood counts are the main ones as they help to determine the normal and pathological processes occurring in the body of animals[4-8].

The hematological parameters of cows, depending on their living conditions and their feed while using biogenic immunomodulators have not been fully studied. Although there are large studies and databases covering many causes of blood cell counts and their changes (depending on the physiological condition of the animal), there is insufficient data on blood cells in comparison to drugs derived from chicken embryos and plants. In this regard, research into new drugs that can correct bovine dry and postpartum metabolism is essential[9-11].

MATERIALS AND METHODS

Ethical statement

The experiment was reviewed at the committee of Ethics of Samara State Agrarian University, under protocol ¹ 1 (13th january 2022) and all experiments were carried out strictly under laws of the Russian Federation "On the responsible treatment of animals and on amendments to certain legislative acts of the Russian Federation" (Federal Law from December 27, 2018 ¹ 498-FZ)

Experiment design

Researches were conducted on Holstein cows, average body weight of 600 kg at the dairy farm Krasniy Klyouch ('Êðàñíûé Êëþ÷' in Russian), an OJSC (Open Joint Stock Company) located in the Samara Region of the Russian Federation. To determine the degree of influence of the STEMB drug and the immunomodulatory substance on morphological, biochemical, immunobiological and enzymic blood values, three groups of 20 cows with similar analogical properties were formed (experimental group-1, experimental group-2, experimental group-3). All animals were 8 months pregnant which was determined through rectal examination, ultrasound and according to the initial documents of insemination logs. All the animals were given the same living conditions, feed and maintenance routine.

Materials

STEMB (Provided by the company "Biomodul", Stavropol, Russia) presents itself as an effective bio-stimulating drug obtained from chicken embryonic tissue. It provides imunostimulating, adaptogenic, bacteriostatic and pathogenetic-supressing effects, and also stimulates the organism's vital functions due to changes in the metabolism and in turn acts on the hematopoietic and enzyme system.

Immunopharm (Provided by Saratov Veterinary Research Institute, Saratov, Russia) – An immunomodulatory substance containing Formaldehyde (0.07-0.24 %) as active substance, Sodium Chloride (0.90-0.95 %) and distilled water. According to the author of the patent RU ¹2077882 À61KÇ1115 (Russian: ÐÔ ¹2077882 À61ÊÇ1115), the substance provides the ability to stimulate the vital functions of the organism by impacting cellular immunity, hematopoiesis, metabolic energy processes and the immunobiological status of the body. The experimental study on the use of the immunomodulating agent for the prevention of birth and postpartum complications in cows was carried out according to the following research scheme[12].

Method of analysis

The animals in the first experimental group were injected with the STEMB reagent thrice, subcutaneously into the neck region at a dose of 0.075 ml per 1 kg of body weight, starting at 30 days before calving at an interval of 7 days between injections. The animals in the second experimental group were administered with Immunopharm intramuscularly 30 days before delivery, thrice at intervals of 7 days at a dose of 6.0 ml per cow[13]. Animals in the third experimental group were administered with the drugs STEMB and Immunopharm combined and at the same dosages and injection scheme in the two previous groups. Both solutions were shaken well and brought to room temperature before use.

To determine the hematological indicators of the group of cows studied, we conducted a blood test on the 15th day after calving. During this process, the morphological, biochemical and immunobiological blood values were determined at the hematology laboratory of the Samara State Agrarian University (ÔÃÁÎÓ ÂÎ Ñàìàðñêèé ÃÀÓ) and in the hematology laboratory of Samara State Medical University. All the experimental data were processed in digital form using the variational inference method for the reliability of differences and the Student's t-test criteria, which is used generally in biology and zoo-technology. Microsoft Excel 7 was the software used to arrange data.

The degree of reliability of the processed data is reflected in the corresponding designations

Ð<0.05; Ð<0.01; Ð<0.001. The experiment was reviewed at the committee of Ethics of Samara State Agrarian University, under protocol ¹ 2 (15th November 2021).

RESULT AND DISCUSSION

The blood test performed on cows on the 15ᵗʰ day after calving showed that the value of morphological parameters had inconsistencies in the animals which can be illustrated by the Mean absolute error (MAE).

Depending on the STEMB and Immunopharm scheme used, several variations were observed in the blood profile of cows (Table 1). The hemoglobin content in animals of the first experimental group was 113.67 g/L which was therefore 2.19 g/L greater than the animals in the second experimental group and 3.86 g/L less than the animals in the third experimental group (P<0.01). The content of erythrocytes in the third experimental group where Immunopharm and STEMB were combined was shown to be 6.75 mln/mm³, which was 0.7 mln/mm³ more than when using the Immunopharm separately and 0.46 mln/mm³ more when using the drug STEMB on its own. This seems to indicate an increase in oxidation-reduction reactions in the body of animals in the Third group.

The decrease in the number of leukocytes by 0.23 thous./mm3 and 0.40 thous./mm3 in animals of the first and second experimental groups in comparison with the third experimental group indicated a decrease of the stimulating effect when used separately.

The combined use of the drugs STEMB and Immunopharm significantly increased the platelet content in the third experimental group at 481.53 thous./mm3; which was greater than the first and second experimental group at 81.81 thous./mm3 and 94.88 thous./mm3 respectively and this means that it had a possible positive effect on the regeneration of the mucus lining of the uterus and an increased coagulability of blood (P<0.001).

After analyzing the differential leukocyte count, the number of basophils and neutrophils had no significant differences between the animal groups. The number of segmented neutrophils in the third experimental group were 38.52 % and the monocytes were 5.32 % which is greater than

in the first experimental group (STEMB only, 0.075 ml/kg,thrice with 7 days interval between injections 30 days prior to delivery) and the second experimental group (Immunopharm only, 30 days before delivery, thrice at intervals of 7 days at a dose of 6.0 ml per cow). This indicated that there was an increase in the activity of the cellular factor of the body's defenses in the third group of animals.

Biochemical blood parameters of cows from the experimental groups on the 15th day after calving showed differences in values, depending on the type of drug scheme used (Table 2). An increase in the content of alpha globulins (á-globulin) and gamma globulins (ã-globulin) while having a low content of beta globulin (â-globulin) in the third experimental group indicated an increase in the immune strength of the organism and this can also be confirmed by the Ig A, Ig M and Ig G levels, which were significantly lower in the first and second experimental group as compared to the third group.

In the blood serum of cows of the third experimental group, where STEMB and Immunomodulatory substance were used together, the total protein content was 80.88 g/L, which was 6.08 g/L more than in cows of the second experimental group (treated with Immunomodulatory substance only) and 5. 95 g/L greater than the cows of the first experimental group (treated with STEMB only) (P<0.01). Hence we can conclude that the clotting and infection-fighting abilites of the third group were moderately increased.

There were differences in the content of protein fractions of the animals. The content of Alpha-globulins (á-globulin) in the third experimental group (combined use of both drugs) was 13.84 % which was greater by 1.97 % and 2.14 % as compared to the first and second experimental groups respectively) (P<0.01). This concludes an increase in the defense mechanism of the animal.

Subsequently the content of Gamma Globulins in the third experimental group was 35.26 %, which were 9.20 % and 9.09 % more than the cows of the first and in the second experimental groups (P<0.05). The increased values of gamma-globulins of the third group supported the argument that the defense mechanism was enhanced; as claimed by Filatov A.I.[11].

The glucose content in the third experimental group was 4.01 mmol/L, which was 0.81 mmol/L more than in the first experimental group and 1.03 mmol/L more than in the second experimental group (P<0.05). Therefore, coming to the conclusion greater amounts of energy can be hence obtained from glycolysis, which can be very crucial in the post-partum period.

The study of the immunoglobulins level (Ig A, Ig M, Ig G) in blood serum of the studied groups of cows, depending on the scheme of drugs administration of STEMB and Immunomodulator

Table 1. Morphological parameters of cows on 15th day after calving

Values	Normal values	Groups of animals		
		group-1	group-2	group-3
Hemoglobin, g/L	99.0-120.0	113.67±0.87	111.48±0.39	117.53±0.92[b]
Erythrocytes, mln/mm³	5.0-7.5	6.29±0.70	6.05±0.30	6.75±0.27
Leukocytes, thous./mm³	4.5-12.0	8.25±0.28	8.08±0.32	8.48±0.25
Platelets, thous./mm³	260-700	399.72±9.26	386.65±9.02	481.53±9.76[c]
Leucogram, %				
Basophils	0-2	1.36±0.07	1.35±0.13	1.48±0.09
Eosinophils	5-8	5.04±0.23	4.99±0.12	5.27±0.10
Neutrophils including:				
Metamyelocyte	0-1	0.06±0.01	0.07±0.02	0.02±0.01
Band	2-5	1.04±0.24	1.20±0.08	1.01±0.09
Segmented	20-35	37.98±0.65	37.25±0.29	38.52±0.44
Lymphocytes	40-65	49.60±0.50	50.45±0.37	48.38±0.52
Monocytes	2-7	4.92±0.12	4.69±0.11	5.32±0.16

[a]P<0.05; [b]P<0.01; [c]P<0.001

Table 2. Cows' Blood Chemistry Values on the 15th Day Postpartum

Parameters	Normalvalues	Groups of animals		
		group-1	group-2	group-3
Total calcium, mmol/L	2.51	2.43±0.08	2.42±0.06	2.69±0.05
Inorganic phosphorus, mmol/L	1.48	1.50±0.04	1.48±0.07	1.68±0.08
Alkaline reserve, %CO_2 volume	50.0-62	48.49±0.32	48.52±0.24	49.52±0.38
Carotene, mg/%	0.54	0.45±0.09	0.46±0.05	0.54±0.06
Glucose, mg%	2.0-4.0	3.20±0.03	2.98±0.04	4.01±0.02[a]
Total protein, g/L	60.0-85.0	74.93±0.63	74.80±0.25	80.88±0.52[b]
Protein Fractions %:				
Albumins	30.0-50.0	43.62±1.42	43.78±0.61	48.50±0.48[b]
Globulins,% including				
Alpha Globulins	12.0-20.0	11.87±0.27	11.70±0.12	13.84±0.10[b]
Beta Globulins	10.0-16.0	12.42±0.30	12.40±0.14	12.35±0.15
Gamma Globulins	25.0-40.0	26.09±0.28	26.17±0.32	35.26±0.27[a]
Immunoglobulins, mg/dL				
À	191.37	164.14±4.07	161.82±2.54	189.83±3.03[b]
M	120.0	137.19±2.86	137.42±2.13	159.54±2.46[b]
G	1209.1	1193.17±9.16	1188.67±9.12	1242.31±3.17[b]
ALT, u/L	60-80	82.14±3.07	81.93±3.66	76.14±2.13[a]
AST, u/L	80.0-100.0	92.45±4.07	93.16±2.13	81.23±3.18[a]

[a]P<0.05; [b]P<0.01; [c]P<0.001

was of great importance for justifying of the obtained results. The combined administration of the drug STEMB and Immunopharm (Third group) significantly (P<0.01) increased the content of Ig A by 25.69 mg/dl; M - by 22.35 mg/dl; G - by 49.14 mg/dl when compared with the independent administration of the STEMB drug (First group), and the when compared to the second group (Immunopharm only) increased the content of Ig A by 28.01 mg/dl; Ig M - by 22.12 mg/dl; Ig G - by 53.64 mg/dl, hence indicated an increase in the of the immune status of the organism of cows in the third experimental group. The content of immunoglobulins M and G was significantly higher (P<0.01) than in the blood of cows of the first and second experimental groups.

The ALT content in the third group where both drugs were combined together was 76.14 units/L, hence restored to the normal values. When each drug was used separately, in the first and second group, an increase in the ALT enzymes was observed (P < 0.05). The AST enzyme content of the third group was 81.23 units/L, lower than that of the first and second group by 11.22 units/L and 11.93 units/L respectively (P < 0.05).

The research results have shown us that hematological parameters of the cows, depending on their living conditions, their feed, and use of immunostimulating biogenic drugs, were not fully studied. The parturition and postpartum complications are multifaceted. The authors first studied the preventive properties and dosages of the combined use of drugs STEMB and Immunopharm and concluded that they have pathogenesis-suppressing, immune-stimulating and regenerative properties from the comparative perspective of the drugs used.

The hematological parameters of cows in experimental groups of animals were variable.

The combined use of STEMB and the immunomodulator increased the total protein, alpha globulins, immunoglobulins A, M and G. There was also a rise of monocytes, which confirmed the presence of phagocytosis. The boost in hemoglobin also indicated an increase in redox processes in the body of this group of animals and the optimality of the combined use of the drugs which helped to ensure the normal bovine metabolic processes. The decreased indicators of ALT and AST enzymes showed that the use of these drugs helped to

normalize liver function in comparison with the separate use of these drugs. Our data were consistent with the opinion of S.V. Shabunin[2], O.S. Shatalina[11] whereby the increase in immunity stimulation, homeostasis and resistance as well as the strengthening of the cow's liver provides an increase in the disease-prevention effectiveness of the integrated use of the drugs.

For the first time, experimental data was obtained for morphological and biochemical values in the cows based on combined used of STEMB agent injected subcutaneously at dosage of 0.075 ml per 1 kg of body weight, and the immunomodulator injected intramuscularly at a dosage of 6ml per cow, both solutions injected thrice at intervals of 7 days starting at 30 days before delivery.

According to the results, the combined use of STEMB agent and Immunomodulatory substance can be recommended for use as a preventive therapy for bovine postpartum complications.

CONCLUSIONS

The combined use of STEMB (0.075 ml/kg, Subcutaneously, thrice with 7 days interval, 30 days before calving) and Immunomodulatory substance (6 ml/cow, Intramuscularly, thrice with 7 days interval, 30 days before calving) is optimal and ensured an improvement in the blood cell composition, an increase in the monocytes, segmented neutrophils, hemoglobin, total protein, alpha globulins, Ig A, Ig M, Ig G, and a decrease in ALT and AST enzymes values as compared to the separate use of these drugs which can be seen in the data obtained on the 15th day post-partum. We concluded that the state of the body's immune system was improved using biogenic drugs with immunomodulating, regenerative, and suppressing pathogenesis, with the ability to influence enzyme systems.

The combined administration of the drugs STEMB and Immunopharm for the prevention of parturient and post-parturient complications is a contribution to veterinary science specifically veterinary obstetrics and veterinary hematology of high-yielding dairy cows. It will help develop new methods for the rational use of biogenic drugs of animal and plant origin while taking into account

the age of animals, breeds, and the level of milk productivity.

REFERENCES

1. Shabunin S. V., Savchenko L., Nezhdanov A. G., Mikhalev V. I., Pasko N. V., Koshchaev A., Interferontau in the blood of cows as an indicator of the emerging pregnancy. Reproduction in Domestic Animals. 2019; Ò.54.¹S3:112-113.

2. Sineva A. M., Lukina V., Nezhdanov A. G., Shabunin S. V., Safonov V., Lobodin K. A. Indicators of protein metabolism and serum transferase activity in holstein breed cows in the dynamics of the postpartum period and lactation. Reproduction in Domestic Animals. 2019; Ò.54.¹S3:121.

3. Lisenko A., Sineva A. M., Safonov V., Nezhdanov A. G., Shabunin S. V., Lobodin K. A. Nitric oxide levels in cows after parturition – influence on folliculogenesis. Reproduction in Domestic Animals. 2019; Ò.54.¹S3:122.

4. Areshidze D. A., Timchenko L. D., Kozlova M. A., Semin I. A., Rzhepakovsky I. V., Piskov S. I., Vakulin V. N. Influence of biologically active substance "STEMB" on a morphofunctional condition of a liver and kidneys of rats at an experimental hepatorenal syndrome. Journal of Pharmacy and Nutrition Sciences. 2015; Ò.5.¹3:186-193.

5. Koba I. S., Lysenko A. A., Koshchaev A. G., Rodin I. A., Shantyz A. U. Effective treatment of chronic endometritis in cows by florinazol preparation. Indian Veterinary Journal. 2017; Ò.94.¹11:15-18

6. Safonov V., Nezhdanov A. G., Sineva A. M., Lisenko A., Lobodin K. A., Lukina V. The trace element status of lactating holstein breed cows during the resumption and depression of ovarian ovulatory function after parturition. Reproduction in Domestic Animals. 2019; Ò.54.¹S3:135-136.

7. Baimishev M. H., Eremin S. P., Baimishev K. B., Baimisheva S. A. Blood indicators of dry cows before and after administration of a drug STEMB. Asian Pacific Journal of Reproduction. 2019; Ò.8.¹1:25-29.

8. Filatov A. I., Ushakova L., Eremin S. P., Musidrai A. A., Anipchenko P. S. Prevention of postpartum diseases in cows. Reproduction in domestic animals. 2018; 53¹S2:133-134.

9. Shabunin S. V., Nezhdanov A. G., Safonov V., Lobodin K. A. Oxide-antioxidant status of dairy cows under postpartum pyo-catarrhal metritis. Reproduction in Domestic Animals. 2016; Ò.51.¹S2:140.

10. Konopeltsev I., Baymishev H. B., Batrakov A., Shiryaev G., Anipchenko P., Nikolaev S. New method of gonadorelin application for treatment of cows with follicular cysts. Reproduction in Domestic Animals. 2018; Ò.53.¹ S2:151-152.

11. Shatalina O. S. The association between blood group and reproductive performance in cattle. Agricultural science. 2018; 53(2):309-317.

12. Laskavy V. N., Nochevny V. Ò., Violin B. V. Formic aldehyde as native immunomodulator. Agrarian Science. 2006; ¹ 8:24-29.

13. Baimisheva S. A., Eremin S. P., Petuhova E. I. Efficacy of doses of Immunopharm for the prevention of postpartum complications in cows. Contribution of young scientists in agrarian science. 2018; 180-182.