



Immune Status of the Risk Group Calves within the Neonatal Period and its Correction

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ABSTRACT:

Introduction. The number of neonatal calves with morphofunctional insufficiency, which should be referred to risk group, increased because of high-productive cow breeding in Russia. Hypotrophy is widely spread among the number of inborn development disorders and intranatal asphyxia is widely spread among the acquired ones.

The mentioned pathologies are accompanied by apparent humoral and cellular defence level decrease in neonatal calves. It is also accompanied by the imbalance among key mediators, produced by various types of cells. This work is devoted to study of various immunocorrective agent impact on the immune system formation in calves of the risk group.

Study Materials and Methods. 32 neonatal calves with different levels of morphofunctional development were selected and divided into 4 groups. The calves of the 1st group were treated by probiotic “Prolam”, containing indigenous microflora; the calves of the 2nd group were treated by synthetic hexapeptide “Immunofan”; the calves of the 3rd group were treated by probiotic together with oligopeptide; the 4th was a control group (therapeutic agents were not used). The nonspecific immunity indexes (BSBA, BSCA, BSLA, LPA, PN, PI), concentration of IL-1, IL-2, IL-4, IL-8, IL-10, TNF- α , synthesized by leukocytes after a twenty-four-hour stimulation by *Staphylococcus aureus* or *Salmonella dublin*, gene expression TLR-2, TLR-4, IL-1 β , IL-2, IL-4, IL-8, IL-10, IL-12p40, TNF- α , INF- γ , TGF- β were studied in calves.

Results. The studies stated that immunotherapeutic agent application at the moment of immune system formation in neonatal calves within colostrum period promoted the correction of immune response to antigens in the changed living conditions. At the same time the direction of the immune reaction development depends on the origin of the immune drug and its effect. The combination of agents, possessing various application points, led to immune response intensity decrease.

Conclusion. The use of the immunocorrective agents in neonatal animals promotes more adaptive immune response formation. Developing and improving immunodeficiency treatment and prophylaxis schemes, applying immunotherapeutic agents of various origins to animals, it is necessary to take into account the mechanisms of their effect and application points.

Key words: calves, intranatal asphyxia, hypotrophy, immunocorrection, “Immunofan”, “Prolam”, immune system, immunity, cytokines.

Introduction.

Production and preserving young healthy farming animals is one of the most actual problems, a successful solving of which greatly determines animal husbandry efficiency. The key role in forming nosological profile diseases in newborn calves during colostrum and subsequent growth periods belongs to perinatal pathology (D.D. Gamboyev, 1997, G.A. Tumilovich, V.V. Malashko, 2008, Y.N. Alekhin, 2013 et al.).

The calves with morphofunctional disorders of antenatal (congenital hypotrophy) and intranatal (asphyxia) origins belong to the risk group (N.V. Valiyev, 1974, V.F. Lysov et al., 1988).

A lot of scientific works are devoted to the studies of the mentioned above pathologies. Those studies uncovered their aetiology and pathogenesis. It is shown that congenital hypotrophy is a fetus's compensatory-adaptive change, determined by the lack of oxygen, nutrients, and bioactive substances supply or by the assimilability derangements of them (N.V. Valyev, 1974, B.M. Anokhin, 2002, Y.N. Alekhin, 2013). Intranatal asphyxia appears in case of fetoplacental blood circulation acute insufficiency or stoppage under the impossibility of lung respiration starting (Y.N. Alekhin et al., 2012, 2013).

The study of the immune status of calves with congenital hypotrophy (A.G. Shakhov et al., 2013) showed that fetal development disorder is accompanied by apparent humoral and cellular defense retrogression and imbalance among key mediators, produced by various types of cells.

Lower ratios of nonspecific humoral (blood serum bactericidal activity – BSBA, blood serum lysozyme activity – BSLA, blood serum complementary activity – BSCA), cellular (leucocyte phagocytic activity – LPA, phagocytic number – PN,

phagocytic index – PI), and specific immunity are detected during the colostric period that indicates a more likely possibility of infection emergence and development. Against the background of antigenic influence increase noticeable IgG retrogression in blood in comparison with healthy developed animals was marked as a result of its (IgG) increased expense.

The studies of gene expression in leucocytes and cytokine concentration in blood serum testify that the calves with hypotrophy syndrome during the neonatal period have acute inflammatory reaction with high concentration of proinflammatory cytokines and lowered concentration of cell and antibody (B-cell) response regulators that can lead to reserve supply depletion of immunocompetent cells and inability to react adequately on the infectious agents penetration into the calves' organisms.

Morphofunctional state disorder was one of the causes of a mass diarrhea infection among calves with hypotrophy and it also appeared as a result of organism adaptive changes that revealed in apparent natural resistance factors suppression. It became a releasing mechanism of gastrointestinal and subsequent respiratory pathology emergence.

Lower ratios of nonspecific humoral (BSBA, BSLA, BSCA), cellular (LPA, PN, PI) immunity, the levels of Ig G, M in blood serum and colostric antibody titers to the bacterial (*Escherichia coli*) and viral (diarrhea virus- mucous membrane disease – DV-MMD) antigens were revealed, studying the immune status of calves that had asphyxia in comparison with the same indexes of the calves without hypotrophy. Low colostrum protein digestibility was also detected, that indicates higher susceptibility of their organisms to the infection emergence and development.

The results of cytokine genes (participating in inflammatory, cell-mediated and humoral response) expression ratio study showed that the calves, which suffered asphyxia, had increased proinflammatory cytokine ratio – Interleukin-1 during the whole neonatal period. The cytokine expression of the calves without hypotrophy had a stable ratio. Cytokine ratio responsible for nonboosted (interferon-gamma and TNF), cellular (interleukin-2, interleukin-12) and humoral (interleukin-4) immune responses was higher in calves without

hypotrophy. The expression of interleukin-2 that is the growth factor and T-lymphocyte differentiation factor, activating macrophages and stimulating lymphokine secretion, and interleukin-4 responsible for lymphocyte proliferation, macrophage activation and antibody production by B-lymphocyte was higher among them in comparison with the animals which suffered asphyxia.

The obtained data show that the calves, which suffered asphyxia, had slower formation of classical cellular and humoral responses during the colostrum period and had higher risk of illness emergence, its transformation into chronicity (A.G. Shakhov et al., 2013).

Thus hypotrophy calves and animals, which suffered intranatal asphyxia, had immune system formation disorders. Immune system plays the main role in antiviral and antibacterial organism defense, its potential is genetically formed during the intrauterine growth period (Y.S. Voronin et al., 2002, J. de Groot et al., 2005).

Various agents and methods that are divided into three groups are offered for the immunocorrective therapy (D.K. Novikov et al., 2002).

1. Biological, originated from cells and tissues of the living organisms (animals, humans, microbes, plants).
2. Chemical (natural and synthetic).
3. Physical (radial energy, ultrasound, magnetic field, etc.).

The aim of the research is to study various immunotherapeutic agent influence on the immune status of calves with different morphofunctional development.

Study Materials and Methods.

32 newborn calves were chosen for the study. After the birth the calves were kept in individual houses and were fed on mother's colostrum (milk) during three days, then they were given the milk turned sour (formic acid). The animals weren't exposed to drug therapy.

The calves were divided into 4 groups (n=8, 4 – with intranatal asphyxia, 4 – with congenital hypotrophy).

«Prolam» and «Immunofan» were used as immunocorrective agents. Probiotic «Prolam» includes viable strains of lactic-acid bacteria *Lactobacillus delbrueckii subsp. bulgaricus* (B-5788), *Lactobacillus acidophilus* 43c (B-3235) in amount of

not less than -5×10^7 cfu/cm³, lactic-acid streptococci *Lactococcus lactis subsp. lactis* 57₄ (B-3145), *Lactococcus lactis subsp. lactis* 170₄₋₅ (B-3192)- 5×10^7 cfu/cm³, *Bifidobacterium animal* 8₃ (AC-1248)- 1×10^7 cfu/cm³, and additives such as water, beet molasses, milk or lactoserum.

Chemical immunocorrective agent “Immunofan” is a synthetical hexa-peptide arginyl- α -aspartyl-lysyl-valyl-tyrosyl-arginine and it includes 0,005% solution of reactant, aminoacetic acid, and NaCl.

Animals of the first control group (intact) were not subjected to treatment. Animals of the second group per os with colostrum were daily treated by probiotic “Prolam” in a dose *Lactobacillus delbrueckii subsp. bulgaricus* (B-5788), *Lactobacillus acidophilus* 43c (B-3235) in amount not less than $-3,5 \times 10^8$ cfu/cm³ (colony forming units), lactic streptococci *Lactococcus lactis subsp. lactis* 57₄ (B-3145), *Lactococcus lactis subsp. Lactis* 170₄₋₅ (B-3192)- $3,5 \times 10^8$ cfu/cm³, *Bifidobacterium animal* 8₃ (AC-1248)- 7×10^7 cfu/cm³ within 7 days. Calves of the third group were intramuscularly treated by “Immunofan” in a dose of 50 micrograms of reactant on one animal three times with a 24 hour interval beginning with the first day of life. Calves of the fourth group got “Prolam” with “Immunofan” according to the schemes of the second and third groups.

The animals underwent clinical observation within 10 days. Their immune status was studied before and after drug application. The whole blood, blood serum, and plasma of the calves were the materials for study.

Bactericidal, lysozyme, and complementary activities were determined by the method of E.S. Voronin et al. (2002). For BSBA study, 1 ml of serum and 0,1 ml of *Escherichia coli* twenty-four-hour broth culture were added into 4,5 ml of Hottinger Broth. Microorganism culture was added only into control samples. The content of tubes was thoroughly mixed. Optical density (OD₄₉₀) was measured in 2 ml of the mixture. The mixture left in tubes was incubated in a thermostat at 37°C within 3 hours and then optical density was measured again. The activity of control samples was defined in units of the optical density growth inhibition in comparison with the test samples.

For BSLA detection, 0,4 ml of 0,06 M phosphate buffer (pH 7,2-7,4) and 2 ml of *Micrococcus lysodeicticus* microbial suspension with optical density 0,215 OD₅₄₀

were added to 1 ml of blood serum. The control contained 0,5 ml of phosphate buffer and 2 ml of microorganism suspension, standard samples contained 0,4 ml of phosphate buffer, 0,1 ml of lysozyme solution with the known activity, and 2 ml of micrococcus suspension. The samples were incubated 30 minutes at 37°C and the optical density was measured (OD₅₄₀). Lysozyme activity was expressed in micrograms/ml.

For BSCA detection 0,1 ml of blood serum was added into 5,9 ml of 0,89% NaCl solution. Control samples contained 5,9 ml of bidistilled water. The samples were incubated 30 minutes at 37°C, control at 56°C for complement system inactivation. 4 ml of prepared and heated within 30 minutes at 37°C hemolytic mixture, containing 2,5 % of sheep red (blood) cell solution and rabbit hemolytic serum, were added into all the tubes after incubation. The samples were placed for 30 minutes into water bath at 37°C, centrifugated at 3000 rpm within 10 minutes. Supernatant optical density was measured at OD₅₂₀. The activity was expressed as a percentage ratio of test samples optical density to control samples. For LPA, PN, and PI detection 0,5 ml of *Staphylococcus aureus* (1,5 billion microorganisms/ml) suspension inactivated in water bath at 100°C within 60 minutes were added to 0,5 ml of blood stabilized by heparin (5000 units/ml). The samples were incubated within 30 minutes at 37°C, the smears were made on the degreased glass slide in three replications, fixed by methanol and Giemsa staining was used.

Leukocyte phagocytic activity was expressed by active leukocyte (phagocyte) percentage in 100 counted neutrophilic leukocytes.

Phagocytic index is an average number of phagocytized microbes per one active leukocyte. PI was detected by the way of dividing the number of phagocytized bacteria on the number of active neutrophils.

Phagocytic number was detected by the ratio of phagocytized bacteria to the total number of neutrophils.

For the expression detection of the receptor and cytokine genes, participating in the immune response (TLR-2, TLR-4, IL-1, IL-2, IL-4, IL-8, IL-10, IL-12p40,

TNF- α , γ -INF, TGF- β), 0,5 ml of *Staphylococcus aureus* or *Salmonella dublin* (1,5 млрд. мк/мл) inactivated in water bath at 100°C within 60 minutes were added to 0,5 ml of blood stabilized by heparin (5000 units/ml). Common RNA was separated from the samples after 24 hours of incubation at 37°C using RIBO-zol-A kit (InterLabService, Moscow, Russia), complementary DNA was synthesized with the aid of PEBEPTA-L (InterLabService, Moscow, Russia). The formed complementary DNA in the concentration of 200 micrograms/ml was used in PCR with primers specific for the required genes. Specific reactions were checked by electrophoretic separation of the reaction products. The expression ratio was estimated with the aid of Amplifier DT-96 (D. C Werling et al., 2004, R. E. Sacco et al., 2012).

Quantitative determination of cytokines (IL-1, IL-2, IL-4, IL-8, IL-10, TNF- α) was made in blood plasma by immunoenzyme method according to the kit instructions (Vector-Best, Novosibirsk, Russia). Whole blood was centrifugated at 8000 rpm within 10 minutes after a twenty-four-hour activation by the microorganisms, 0,5 ml of supernatant were placed into clean tubes and kept at -20°C till the beginning of the studies.

Results.

The neonatal calves got from the cows with prolonged labors had intranatal asphyxia symptoms within the first hours of life: depressed condition with apparent cyanosis of visible mucous membranes, hyperemia of gums, depressed appetite and sucking reflex, significant increase of pulse ($136,0 \pm 5,1$ ictuses) and respiratory ($38,0 \pm 2,0$ per minute) rates. Independent standing posture was registered only in $6,5 \pm 2,0$ hours.

Permanent asphyxia symptoms such as prolonged tachycardia and dyspnea appeared in 3-4 and 6-7 days respectively.

Apparent inhibition of physiologic reflexes, tendency to hypothermia, moderate tachycardia, tachypnea, respiratory volume decrease, prolonged imbalance of respiratory phase were revealed in calves with hypotrophy.

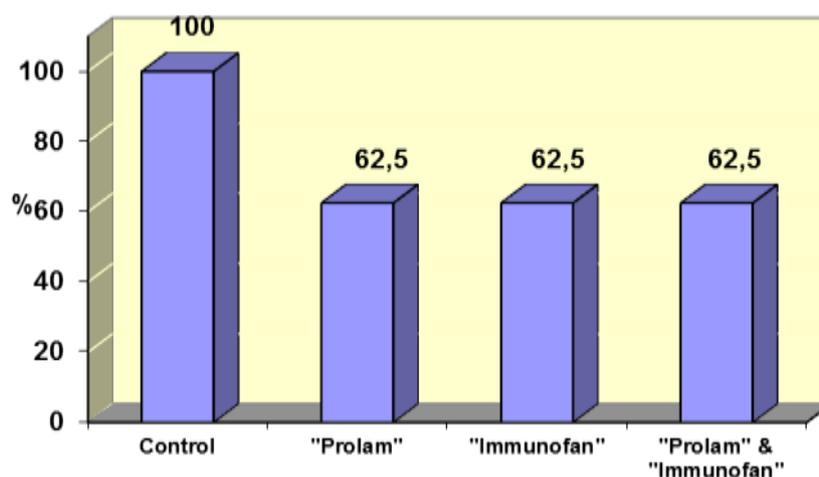
Calves with the normal level of development had the mentioned above indexes within a reference range for healthy animals.

Diarrhea syndrome was registered in animals of all the groups but the number of ill animals, severity of onset and the duration of the disease were different within the colostric period.

The incidence of gastrointestinal diseases was 100% with the average duration of 6,4 days in the control group. Enteritis form of colibacillosis was registered in 4 calves (50%). Rotavirus genome was detected in 2 animals (25%) and the disease had a severe form. The coronavirus genome was detected in all the animals in 9-10 days.

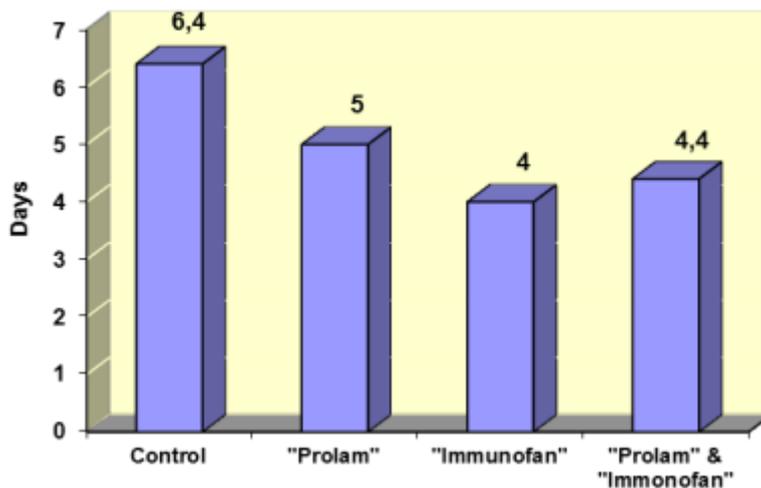
Gastrointestinal diseases were registered in 62,5 % of cases with an average duration of 5 days in the group with calves, which were implemented combined probiotic "Prolam". Enteritis form of colibacillosis was registered in 2 animals (25%). The rotavirus genome was detected in 2 calves (25%) within the first day, the disease was severe.

Figure 1. The risk of gastrointestinal diseases in calves of the risk group



Benign gastrointestinal diseases were registered in 62,5 % of cases with an average duration of 4 days in the group with calves, which were implemented "Immunofan".

Figure 2. Average duration of the diarrhea syndrome in calves of the risk group



Benign gastrointestinal diseases were registered in 62,5 % of cases with an average duration of 4,4 days (Fig. 1-2) in animals, which were simultaneously implemented probiotic and immunomodulatory drug.

As a result of studying the immune status it was stated that antigenic impact of the environment on the organism of neonatal animals led to BSBA reduction to 12,4%, insignificant reduction of the circulating phagocyte number and their activity (PN to 22,6 and PI to 20,6%) and BSCA 14 times reduction in 7 days in the calves of the control group.

The increase of cellular (LPA to 22,6%, PN to 23,7% and PI to 10,8%) and humoral links (BSBA to 7,7%) ratios of nonspecific defense along with 2,2 times BSCA decrease took place in calves after implementation of "Prolam". The prescription of "Immunofan" provoked similar changes that were more expressed: LPA increase up to 14,3%, PN 1,7 times increase, PI 1,5 times increase. It proved high phagocytic and bactericidal activities of blood serum up to 15,1% and 1,7 times complementary activity decrease.

LPA, PN, and PI, BSBA increase along with BSCA decrease proved the activation of the humoral and cellular links of the nonspecific resistance.

The calves, which were treated by "Prolam" in combination with "Immunofan", had the decrease in nonspecific resistance ratios – BSBA to 6,9%, BSCA 3,5

times and BSLA to 12,5%, PN to 12,9 %, it was connected with various drug impacts on the immune systems of the neonatal animals (tab. 1).

Table 1. Immune status ratios of the calves

Drugs	BSBA, %		BSCA, % haemolysis		BSLA, micrograms/ ml		LPA		PN		PI	
	Age, days											
	1	7-10	1	7-10	1	7-10	1	7-10	1	7-10	1	7-10
“Prolam”	71,1±	76,6±	10,8±	4,9±	1,8±	1,7±	77,2±	88,5±	5,9±	7,3±	7,4±	8,2±
	6,08	6,17	3,4	1,11	0,08	0,08	1,85	1,25	0,64	0,71	0,6	0,81
“Immunofan”	74,2±	85,4±	17,1±	10,2±	1,8±	1,7±	76,7±	87,7±	4,7±	8,2±	6,1±	9,2±
	1,2	2,97	1,18	1,31	0,08	0,08	1,43	2,15	0,11	0,77	0,14	0,69
“Prolam” “Immunofan”	76,5±	71,2±	14,7±	4,2±	1,6±	1,4±	77,2±	89,2±	6,02±0	6,1±	7,8±	6,8±
	1,93	1,02	2,61	0,45	0,01	0,21	3,26	1,35	,47	0,55	0,49	0,5
Intact	77,3±	67,6±	15,4±	1,1±	1,6±	1,6±	78,6±	72,5±	6,2±	4,8±	7,8±	6,2±
	2,19	2,3	0,3	0,13	0,05	0,15	3,81	1,25	0,75	0,25	0,64	0,33

Thus the use of immunocorrectors promoted the maintenance of the natural resistance ratio within the adaptation period of the neonatal calves to the new conditions and immune system forming. The animals, which were treated by immunocorrective agents, had higher ratios of the nonspecific defense than the calves of the control group (BSBA to 5,6-26,3%, BSCA 3,8-9,3 times, LPA to 17,4-20,0%, PN to 21,4-41,55%, PI to 8,1-32,6%).

“Prolam” and “Immunofan” separate and aggregated immunocorrective impacts were also estimated by the drug influence on the cytokine gene synthesis and expression by leukocytes (white (blood) cells) stimulated by *Staphylococcus aureus* and *Salmonella dublin*. It is stated that α -TNF concentration decreased in calves of all the groups in 7 days, along with this the animals, which got “Immunofan” and “Prolam”, had the minimum concentration of it and the calves, which got the combination of them, had a lower decrease of the specified

mediator ratio though its amount was lower than in calves of the intact group (tab. 2).

By the fixed time the animals, which got “Prolam”, “Prolam” in combination with “Immunofan”, and control group had the decrease in proinflammatory cytokine discharge – interleukine-8 responsible for neutrophil stimulation that proves insufficient immune reaction to antigenic impact.

In calves, which got “Immunofan”, in contrast to animals of other groups the impact on *Staph. aureus* and *S. dublin* antigen immunocompetent cells led to potentiation of specified interleukin synthesis that promoted the activation of cellular link of immune system.

Table 2. Cytokine concentration in blood serum of calves at daily leukocyte *Staphylococcus aureus* stimulation (picogram/ml)

Drugs	α-TNF		IL-1		IL-2		IL-4		IL-8		IL-10	
	Age, days											
	1	7-10	1	7-10	1	7-10	1	7-10	1	7-10	1	7-10
“Prolam”	25,3±	0,98±	4,3±	9,5±	61,7±	0	0,91±	2,6±	17,2±	11,3±	3,3±	3,6±
	5,8	0,66	1,79	2,92	25,56		0,38	0,42	4,69	1,87	0,84	0,98
“Immunofan”	18,9±	0	11,5±	17,4±	31,1±	30,9±	1,4±	1,8±	4,8±	16,0±	3,9±	3,7±
	8,61		5,95	4,9	9,06	9,02	0,39	0,08	1,69	2,85	0,14	0,04
“Prolam” “Immunofan”	43,2±	21,3±	0,37±	6,1±	21,3±	10,4±	1,8±	2,4±	33,4±	14,9±	2,5±	4,5±
	2,35	9,77	0,24	4,67	9,94	6,14	0,57	0,23	3,49	1,53	0,9	0,42
Intact	42,3±	26,9±	1,1±	7,1±	18,3±	9,9±	1,8±	1,9±	21,6±	13,5±	2,1±	4,5±
	4,56	8,08	1,03	3,19	8,19	6,71	0,4	0,34	5,31	2,59	1,34	0,37

Significant differences were discovered studying interleukin-1 content at cell stimulation by *Staph. aureus* and *S. dublin* antigens. The *S. Dublin* impact on immunocompetent cells led to synthesis decrease and mediator discharge in 7 days in calves of all the groups, especially in animals after immunocorrectors use. Leukocyte (white (blood) cells) stimulation, on the contrary, led to interleukin-1 discharge increase, the activation of the synthesis of which mostly revealed in

animals, which got “Prolam” in combination with “Immunofan” and in calves of the control group.

The mentioned differences of *Staph. aureus* and *S. Dublin* impact on interleukin-1 synthesis are determined by more apparent foreignness of salmonella antigens that promotes faster transition from inflammatory response to cellular immune response. Interleukin-2 concentration increase in response to stimulation by *S. dublin* cells proves it. Interleukin-2 raises cytological function of T-killers and natural killer cells, production of perforins, participating in lysis of bacterial cells and activating macrophages. Staphylococcus impact led to cytokine discharge decrease in calves of all the groups, except the animals after the application of “Immunofan” providing its amount at the same level.

Table 3. Cytokine concentration in the blood serum of the calves at daily stimulation by Salmonella dublin (picogram/ml)

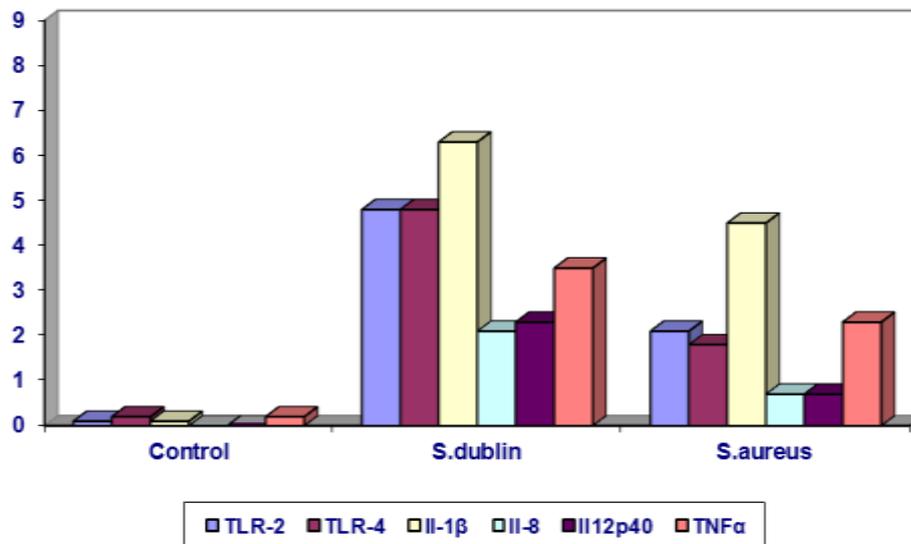
Drugs	α-TNF		IL -1		IL-2		IL-4		IL-8		IL-10	
	Age, days											
	1	7-10	1	7-10	1	7-10	1	7-10	1	7-10	1	7-10
“Prolam”	69,9±	19,2±	47,0±	38,1±	66,3±	79,6±	3,9±	1,7±	47,6±	37,5±	0	19,3±
	0,63	3,74	10,3	7,98	15,2	20,28	0,98	0,12	3,46	3,71		8,18
“Immunofan”	61,0±	16,5±	31,1±	17,6±	97,3±	145,0	1,5±	1,1±	34,5±	42,5±	1,9±	40,6±
	2,86	2,3	4,32	1,95	13,76	±38,2	0,23	0,17	3,39	1,05	1,85	3,65
“Prolam Immunofan”	69,9±	32,1±	69,0±	26,3±	68,7±	55,3±	6,5±	2,5±	48,4±	41,2±	1,6±	4,5±
	3,82	4,37	2,91	1,2	13,88	12,35	0,81	0,34	2,26	2,25	0,81	1,32
Intact	59,1±	46,7±	58,9±	47,9±	45,8±	97,5±	5,0±	4,7±	43,9±	35,5±	8,7±	10,9±
	4,04	4,26	7,26	8,58	9,66	23,4	0,52	1,43	4,78	3,42	3,24	6,38

Interleukin-2 content increase at stimulation by *S. dublin* led to concentration decrease of interleukin-4 which is mostly responsible for humoral immunity and immunoglobulin synthesis by B-lymphocyte. The activation of the discharge of the last in calves after immunocorrection therapy at *Staph. aureus* influence is explained by the higher contact frequency and duration of calves with the microorganism and the presence of specific B-cells and memory cells in blood.

Interleukin-10 content increase in calves of all the groups at bacterial antigens impact is determined by the necessity of animal organism inflammatory response decrease of the reaction on pathogen presence. The stimulation of *Staph. aureus* didn't have similar effect on calves, which got "Immunofan" as at activation of interleukin-8 separation and stable synthesis of interleukin-2. It proves apparent cell reaction (tab. 3).

Studying gene expression of proinflammatory cytokines and immunocompetent cells receptors it was stated that activation of the genes encoding Tull-like receptors 2 and 4 types and proinflammatory cytokines (interleukin-1 and tumor necrosis factor) took place in calves after the application of corrective agents.

Figure 3. Cytokine gene expression in white blood cells (leukocytes) of calves, which were treated by "Prolam", after a daily stimulation by antigens of microorganisms.



The calves, which got "Immunofan", had cytokine gene expression stimulating cell reaction of interleukin-8 and interleukin-12 and it (cytokine gene expression) was more apparent than in animals, which were given "Prolam" and "Prolam" with "Immunofan", that was probably determined by "Prolam" positive effect on intestine microbiocenosis assisting immunity correction directed at cellular immune response decrease and humoral immune response increase (Fig. 3-6).

Figure 4. Cytokine gene expression in white blood cells (leukocytes) in calves, which got “Immunofan”, after a daily stimulation by the antigens of microorganisms.

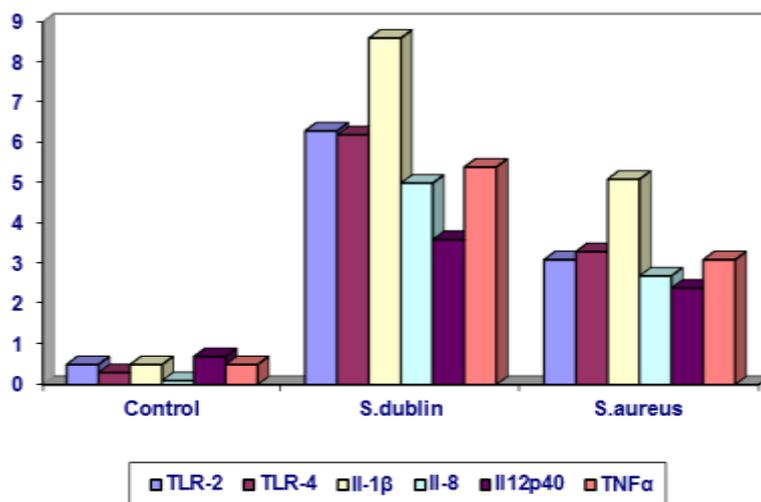
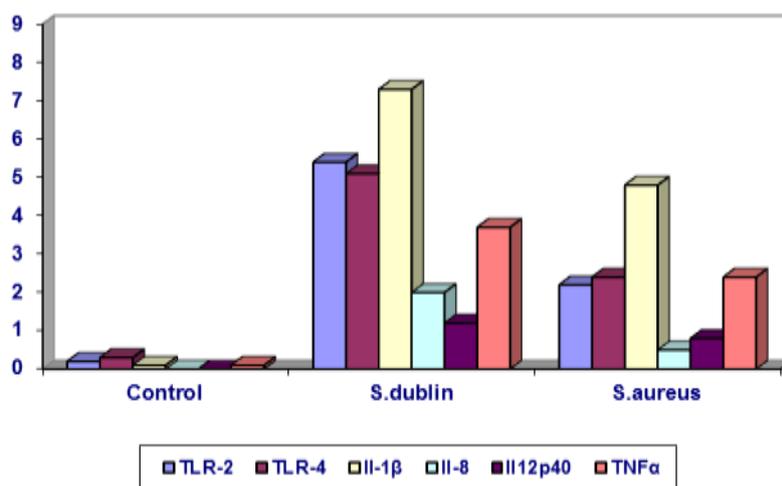
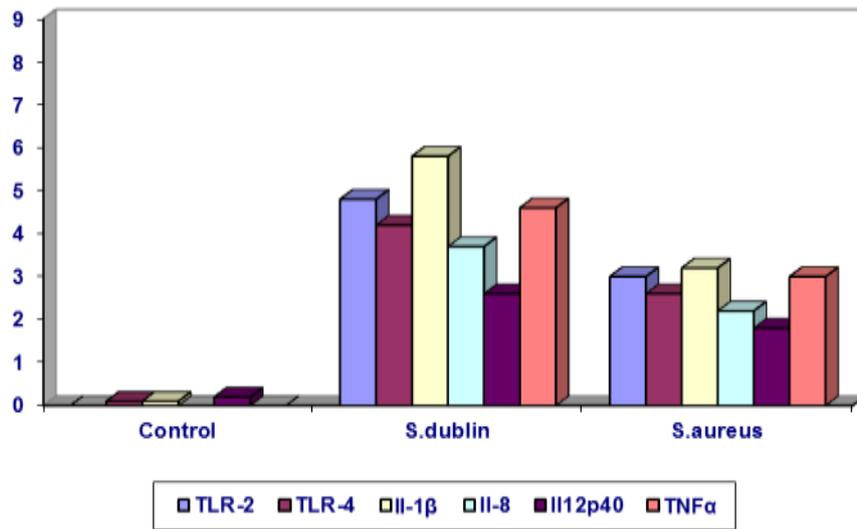


Figure 5. Cytokine gene expression in white blood cells (leukocytes) in calves, which got “Prolam” and “Immunofan”, after a daily stimulation by the antigens of microorganisms.



The proinflammatory gene and receptor expression in calves of the intact group in response to stimulation by antigens of microorganisms was lower than the expression in animals, which were given immunocorrectors. So, it proves a less apparent reaction on antigenic impact.

Figure 6. Cytokine gene expression in white blood cells (leukocytes) in calves of the control group after a daily stimulation by the antigens of microorganisms.



The detected less significant leukocyte response to staphylococcus impact revealing in lower cytokine synthesis and discharge and gene expression at nonspecific resistance factor decrease can lead to inhibition of the immune reaction to the rising antigenic load.

Discussion.

The undertaken researches of stimulation and correction of the immunity formation in calves of the risk group within the colostrum period stated that the use of biological (“Prolam”) and synthetic (“Immunofan”) immunocorrectors promoted better neonatal adaptation to the environmental changes, increased antigenic load and adequate immune response formation.

The use of “Prolam” promoted active neutrophil circulation in bloodstream, their absorbing capacity and blood serum bactericidal action increase that showed nonspecific resistance activation.

Probiotics containing complex of various species of indigenous microflora particularly such as lactobacilli, lactococci, and bifidumbacteria promote not only normal microbocenosis maintenance of open cavities but also have apparent immunostimulating impact. It is stated that separate strains of these microorganisms can significantly increase macrophage phagocytic capacity, potentiate the production of interleukin, interferon, and other mediators, so to increase nonspecific immune resistance (D.S. Yankovskiy, 2005).

On the other hand, it's proved that lactobacilli have a great role in inflammatory process decrease in response to virus infection and protection of intestine epithelium cells and tissues and upper air passages from their damaging action (A.R. Lomax, Calder P.C, 2009, H.F. Rosenberg, 2012). Indeed, S.J Gabryszewski et. al. (2011) showed lactobacillus effectiveness in inhibition of virus-induced inflammation and uncontrolled interleukin synthesis ("cytokine storm") at the expense of granulocyte inflow into the infected tissues.

Our studies stated that leukocyte stimulation by staphylococci and salmonellae in calves, which were given "Prolam", led to proinflammatory cytokine (TNF α IL-8) discharge decrease at rather high expression of their genes, IL-10 synthesis increase connected with necessity of the inflammatory response decrease. However, in contrast to salmonella impact that led to IL-2 level increase stimulation by staphylococcus increased IL-1 β concentration that promoted cytodifferentiation and IL-4 guide development of the humoral response in Th2 path in this case.

The studies of A.Y. Mironov et al. (2011) stated high capacity of lactobacilli to organize biofilms in mucous membranes, their interaction with the intestine immune system. Probiotic bacteria actively participate in forming early barrier against infection, increasing the IgA secretory concentration, phagocytic activity of immunocompetent cells, bactericidal, lysozyme, and complementary activities of blood serum (L.V. Zhichkina, 2010, A.N. Panin, 2011). Certain drugs from probiotics are already offered for immune correction (V.M. Bondarenko, 1998, S. Blum et al., 1999, H.S.Gill et al., 2000).

The other immune correctors and modulators are synthetic peptides that are according to some researchers' opinions appeared as signal molecules as a result of evolutionary track earlier than other mediators of cell-cell interactions (V.H. Khavinson, V.V. Malinin, 2002). The increase of colostrum immunoglobulin absorption intensity, natural resistance formation are observed in neonatal calves, which are given amino acids (L.V. Kharitonov, 2006). Peptide advantage over the other amino acid drugs is in their higher resistance to splitting that is important at parenteral introduction (L.V. Kharitonov, 2012). The use of synthetic immune correctors on the basis of oligopeptides, containing from 2 to 6

amino acids, promotes phagocyte activation, their microbial content, leukocyte adherence increase and active oxygen forms production at contact with opsonized microorganism fragments (D.K. Novikov, 2002, L.V. Kharitonov, 2012).

Our studies stated that immune corrective impact of “Immunofan” in contrast to “Prolam” was mostly directed not at the phagocyte number increase in bloodstream but at their activity and aggressiveness increase. This is proved by blood serum complementary activity decrease that was marked in animals of all the groups. The calves which got “Immunofan”, had lower blood serum complementary activity decrease.

The use of drugs was accompanied by phagocyte activation increase as a result of which the complement was consumed in a lesser degree than in calves of the control group. Higher participation of the complement system’s proteins for bacterial cell lysis and protein penetration into them (this promotes their destruction) is necessary for the unstimulated phagocytes.

The use of “Immunofan” by neonatal calves led to TNF leukocyte synthesis stoppage and IL-1 discharge increase at stimulation by staphylococci but in a lesser degree than in animals treated by “Prolam”. Along with it at a less expressed IL-4 synthesis increase the amount of IL-2 discharged remained at the same level, and IL-8 significantly increased that proves TH1 prevalence in the immune response path and higher macrophage activity. The stimulation of leukocytes by salmonellae as well as in calves, which were treated by “Prolam”, led to IL-10 amount increase, proinflammatory cytokine (TNF- α , IL-1) and IL-10 synthesis decrease. The IL-10 synthesis decrease in animals treated by “Immunofan” was less expressed. They also had higher IL-2 synthesis increase and IL-8 discharge was stimulated. The IL-8 content decreased in calves, which got “Prolam”.

The stimulation of leukocytes by staphylococcus in calves which were treated by “Prolam” in combination with “Immunofan” led to the same changes like in the animals, which were treated by “Prolam”. However, they had TNF discharge decrease in a lesser degree and more apparent IL-8 that can prove a low macrophage activity. Significant increase of synthesized IL-1 content caused comparatively higher increase of IL-10 amount (necessary for stopping

inflammatory process) produced by leukocytes. The transition from Th1 to Th2 immune response path was lower in calves of this group. At incubation of the whole blood by salmonellae the discharge decrease of all the studied cytokines was marked, except IL-10, the synthesis increase of which was lower than in the animals, which got “Prolam” and “Immunofan” separately.

The difference marked in production of the cytokines, stimulated by leukocyte microorganisms at probiotic and synthetic immune corrector application, is explained by the effect of the drugs on various links and factors of the immune system. The microorganisms entering into the “Prolam” composition, participating in intestinal biocoenosis forming, are primary antigens (at early stages of immune system formation) for native leukocytes, stimulating their proliferation and activation that promotes faster development of the immune response when in contact with foreign bacteria predominately in Th2 path. “Immunofan”, being an oligopeptide, mainly plays the role of signal molecule at interaction among immunocompetent cells. At the same time “Immunofan” impact on macrophages/phagocytes leads to their activity and aggressiveness increase without cell number increase that decreases the bone marrow load, increases T- lymphocyte proliferation, stimulates IL-2 production. Therefore its usage promotes the immune response development in Th1 path. The use of “Prolam” and “Immunofan” combination was accompanied by the simultaneous Th1 and Th2 paths activation that led to immune reaction intensity decrease in response to actions of the antigens.

Conclusions.

The studies stated that the application of immunocorrective agents to neonatal calves from the risk group promoted immune response formation more adaptive to antigen impact within the immune system formation period. However, treating the animals by immunotherapeutic agents of different origins it is necessary to take into account the mechanisms of their impact and application points for the immune correction scheme development and improvement.

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