(серологическое) всего маточного стада; 4 Приоритет серологической диагностики на лейкоз; 5 Исключение заражения животных при ветеринарных и зоотехнических манипуляциях; 6 Организация ухода кормления, содержания и разведения скота согласно физиологическим нормам; 7 Проведение комплекса мер по адаптации ввозимого скота.

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Absorption of colostral immunoglobulins in calves with experimental intranal asphyxia

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Abstract

The purpose of this study was to study the causes of violations of absorption colostral immunoglobulin in calves that experienced intranatal asphyxia. Before and after colostrum reception, the blood was examined in 8 healthy and 19 calves with acute intranatal asphyxia. Studies have shown that in newborn calves who have suffered intranatal asphyxia, inhibition of the absorption of colostral immunoglobulins is observed. It was found that acidosis, hypoxia, destruction of membranes, deficiency of buffer base and hypercapnia have the greatest inhibitory effect on the absorption of colostral immunoglobulins. However, some classes of immunoglobulins had a diverse tolerance to pathogenetic factors of asphyxia. The most rapid restoration of absorptive mechanisms was observed in IgG.

Keywords: calves, intranatal asphyxia, colostrum, immunoglobulins.

Аннотация

Целью данного исследования было изучение причин нарушений абсорбции колостральных иммуноглобулинов у телят, испытавших интранатальную асфиксию. До и после приема молозива исследовали кровь у 8 здоровых и у 19 телят, перенёсших острую

интранатальную асфиксию. Проведённые исследования показали, что у новорожденных телят, перенёсших интранатальную асфиксию, наблюдается ингибирование абсорбции колостральных иммуноглобулинов. Установлено, что ацидоз, гипоксия, разрушение мембран, дефицит буферной базы и гиперкапния оказывают наибольшее ингибирующее влияние на абсорбцию колостральных иммуноглобулинов. Однако, некоторые классы иммуноглобулинов имели разнообразную толерантность к патогенетическим факторам асфиксии. Наиболее быстрое восстановление абсорбирующих механизмов наблюдалось у IgG.

Ключевые слова: телята, интранатальная асфиксия, молозиво, иммуноглобулины.

Introduction. Forming optimal levels of colostral immunity is one of the more important aims in the process of neonates' adaptation to extrauterine environment. The level of colostral immunity depends on the intensity of intestinal absorption of colostral immunoglobulins [1]. Many endogenous and exogenous factors have impact on this process. The role of animals' breed and gender [2], quality of colostrum and environmental factors [3] is known to play a role. Immunoglobulin deficiency appears due to a high level of hormonal stress [1, 4, 5] and disturbances of acid-base balance in neonates [6].

According to our data, a deficit of immunoglobulins is observed in 35.0% of newborns after asphyxia intranatalis [7]. However, the causes of this phenomenon are not sufficiently studied, which makes it difficult to find methods for correcting the consequences of perinatal pathology and preventing immunodeficiency. The purpose of this research was to scrutiny the causes of impaired absorption of colostral immunoglobulins in experimental asphyxia in calves.

Material and methods. The experiment was conducted according to the rules of the directive of the European Parliament and the Council on the protection of animals used for scientific purposes [8]. The research included 27 neonate calves obtained from healthy Holstein cows that were kept in individual boxes. Evaluation of parturition and results of neonates' examination were the bases for separating calves into 4 groups: healthy (n=8), experienced Asphyxia intranatalis of first (n=6), second (n=6) and third (n=7) degrees severity, respectively. All the calves did not have any abnormalities and their body weight was normal (32.7 ± 0.8 kg). Then they were placed into individual cages. Calves were given colostrum via catheter in a dose of 5 ml/kg of body weight in 60 minutes after birth. It was the colostrum of the first milking, previously obtained from 7 cows that did not participate in the experiment. It was frozen and stored at a temperature of -18°C . It contained IgG – 75.5 g / L, IgM – 8.2 g / L and IgA – 4.0 g / L.

Blood samples were collected from jugular vein. Intravenous line was established by Seldinger [9] technique during the first blood collection (10-15 minutes after birth). We used a set for establishing intravenous line (B. Braun, Melsungen, Germany). It included needle, guide wire with lengths marking and polyurethane catheter with the length of 200 mm (L=200 ii) and diameter of 1.4 mm (4.5 F). The catheter was washed through by application of 5 ml of a sterile saline solution. Finally, 1 ml of the saline with 10 IU of heparin was injected in the catheter and its orifice was closed by a screw cap after every blood collection. Four blood samples were collected from each neonate (in 10-15 minutes after birth, before giving colostrum and also in 4 and 8 hours after feeding colostrum).

Blood acid-base balance (ABB) and content of gases were analyzed with Rapidlab-840 analyzer (Chiron Diagnostics Ltd, Great Britain). On it we determined: pH, partial pCO₂ pressure (pCO₂), partial oxygen pressure (pO₂) and base deficit (BD).

The content of immunoglobulin classes in serum was estimated by Mancini method (radial immunodiffusion) [10] with the use of species-specific antisera to IgG, IgM and IgA.

Endogenous intoxication level was estimated according to middle (500-5000 D) mass molecules (MMM) and ectoglobular hemoglobin (EGH) contents in serum. To estimate MMM, 0.5 ml of 10% trichloroacetic acid solution were added to 1 ml of plasma. It was centrifuged in 15 minutes, then 4.5 ml of distilled water were added to 0.5 ml of supernatant. The solution was

subjected to photometry using a UV-1800 spectrophotometer (Shimadzu, Japan) at 254 nm in comparison with the control (1 ml of 10% trichloroacetic acid and 29 ml of distilled water). This index is not higher than 0,3 units in healthy animals [11]. The concentration of ectoglobular hemoglobin was studied in blood plasma according to A.V. Karkashev and E.O. Vyachev's method [12].

Membrane receptor complexes of erythrocytes and coefficient of membrane modification by adrenaline (CMMA) were estimated according to the method devised by Alekhin Yu.N. The principle of this method is a quantitative determination of urea haemolysis of erythrocytes in initial samples and even after adding of modifiers (adrenaline, adrenoreceptor blocking agent) [7].

Stabilized blood (heparin) was centrifuged (3000 rpm, 10 minutes) and then plasma was extracted. Equal quantities of 0.9% sodium chloride solution was added to residue and agitated. Then supernatant was removed after centrifugation (3000 rpm, 10 minutes). Equal quantity of 0.9% sodium chloride solution was added to residue and agitated (erythrocyte suspension) (Tab. 1).

Table 1.

Research procedure

Reagents	Tube #	
	1	2
Erythrocyte suspension, ml	0.1	
Propranolol hydrochloride, 0.0025% solution, ml	1.0	
Water bath, 20°C, 5 minutes.		
Erythrocyte suspension, ml		0.1
Sodium chloride, 0.9% solution, ml		1.0
Adrenaline hydrochloride, 0.004% solution, ml	1.0	1.0
Water bath, 20°C, 5 minutes.		
Process solution (1.4 g of urea, 0.213 g of sodium chloride and up to 100 ml of distilled water), ml	3.0	3.0
To agitate. Remains 30 minutes at 18-25°C.		
Centrifuged for 10 minutes at 3000 rpm		
Supernatant is subjected to photometry (540 nm) in cuvette with layer thickness of 10 mm in comparison with distilled water.		
Calculation: CMMA= E2/E1, where E1 – supernatant extinction in tube №1, E2 – supernatant extinction in tube №2.		

Asphyxia intranatalis was detected by assessment of parturition and results of neonates' examination during the first minutes of life [13]. The degree of asphyxia was estimated by calves' examination in 10-15 minutes after birth and their blood test results (Tab. 2).

Table 2

Indices of the calves in 15 minutes after their birth.

Index	Healthy	Asphyxia intranatalis (degree of severity)		
		1	2	3
Mucous membrane color	Pink with cyanotic (blue-tinged) shade	Pink and blue, cyanosis	Blue-pale	Grey, grey-and-white
Pulse, per minute	110-140	140-180	100-180	< 100
pH	7.14-7.22	7.12-7.17	7.05-7.11	< 7.05
pO ₂ , kPa	3.60-4.15	3.30-3.60	2.80-3.29	< 2.8
pCO ₂ , kPa	5.25-5.70	5.30-6.00	6.10-6.50	> 6.5
BD, µm/l	11.30-14.30	13.50-14.50	14.60-17.0	> 17.0

The data were analyzed statistically using unpaired student's t- test to determine the significance of differences between the mean values of the groups and Pearson's chi-squared test (χ^2) to assess the effect of the studied factors on the absorption of immunoglobulins. Data were

expressed as mean $\pm$ standard error ($M \pm m$), coefficient of determination (rd). Statistical significance of $p < 0.05$ was considered significant.

Results. Throughout the experiment, the increase of blood pH and partial pressure of oxygen, as well as partial pressure of carbon dioxide decrease and deficit of bicarbonates, were observed in healthy calves (Tab. 3). MMM, EGH and CMMA indices significantly increased in 4 hours after feeding, but then their decrease was observed. In contrast to healthy calves, neonates that experienced mild asphyxia (1st degree) demonstrated an increase of MMM and CMMA indices throughout the whole experiment and in 8 hours after feeding. The indices increased by 55.8 and 4% ($p < 0.05$), respectively, in comparison with initial level.

The animals after asphyxia of second degree of severity demonstrated blood pH increase in 4 hours after feeding, but it decreased at the end of the experiment. MMM, EGH and CMMA indices throughout the whole experiment increased by 12.1; 25.7 and 10.6% ($p < 0.05$), respectively.

Table 3.

Indices of blood ABB and gases in neonate calves before (upper line), in 4 (middle line) and 8 (lower line) hours after giving colostrum.

Indices	Healthy	Asphyxia intranatalis (degrees of severity)		
		1	2	3
pH	7.200 $\pm$ 0.0003	7.138 $\pm$ 0.0005	7.108 $\pm$ 0.0003	7.010 $\pm$ 0.0003
	7.206 $\pm$ 0.0001*	7.180 $\pm$ 0.0003*	7.114 $\pm$ 0.0003*	6.980 $\pm$ 0.0005*
	7.223 $\pm$ 0.0003*	7.205 $\pm$ 0.0006*	7.109 $\pm$ 0.0006	7.007 $\pm$ 0.0003*
pO ₂ , kPa	4.08 $\pm$ 0.016	3.79 $\pm$ 0.025	3.04 $\pm$ 0.019	2.50 $\pm$ 0.020
	6.49 $\pm$ 0.010*	5.97 $\pm$ 0.016*	4.50 $\pm$ 0.022*	2.97 $\pm$ 0.015*
	6.95 $\pm$ 0.020*	6.68 $\pm$ 0.020*	5.14 $\pm$ 0.015*	3.60 $\pm$ 0.015*
pCO ₂ , kPa	5.49 $\pm$ 0.019	5.80 $\pm$ 0.030	6.00 $\pm$ 0.027	6.08 $\pm$ 0.030
	5.52 $\pm$ 0.021	5.72 $\pm$ 0.019*	5.88 $\pm$ 0.025*	6.00 $\pm$ 0.024*
	5.20 $\pm$ 0.013*	5.50 $\pm$ 0.022*	5.62 $\pm$ 0.015*	5.80 $\pm$ 0.010*
BD, μ m/l	11.0 $\pm$ 0.17	12.4 $\pm$ 0.20	14.5 $\pm$ 0.25	21.0 $\pm$ 0.26
	10.7 $\pm$ 0.10	11.7 $\pm$ 0.13*	13.0 $\pm$ 0.11*	21.9 $\pm$ 0.20*
	10.0 $\pm$ 0.10*	10.8 $\pm$ 0.20*	11.6 $\pm$ 0.17*	22.7 $\pm$ 0.31*
MMM, Units	0.29 $\pm$ 0.001	0.36 $\pm$ 0.003	0.38 $\pm$ 0.003	0.48 $\pm$ 0.003
	0.31 $\pm$ 0.002*	0.38 $\pm$ 0.003*	0.42 $\pm$ 0.005*	0.55 $\pm$ 0.005*
	0.29 $\pm$ 0.001	0.35 $\pm$ 0.005	0.46 $\pm$ 0.005*	0.63 $\pm$ 0.005*
EGH, g/l	0.57 $\pm$ 0.002	0.43 $\pm$ 0.003	0.70 $\pm$ 0.006	1.03 $\pm$ 0.008
	0.60 $\pm$ 0.004*	0.59 $\pm$ 0.005*	0.73 $\pm$ 0.005*	1.25 $\pm$ 0.010*
	0.58 $\pm$ 0.002*	0.67 $\pm$ 0.005*	0.88 $\pm$ 0.005*	1.41 $\pm$ 0.008*
CMMA	1.20 $\pm$ 0.031	1.27 $\pm$ 0.040	1.42 $\pm$ 0.018	1.48 $\pm$ 0.020
	1.33 $\pm$ 0.020*	1.30 $\pm$ 0.025	1.45 $\pm$ 0.021	1.54 $\pm$ 0.041
	1.30 $\pm$ 0.027*	1.32 $\pm$ 0.024	1.57 $\pm$ 0.026*	1.60 $\pm$ 0.045*

*- $p < 0.05$ significant difference from initial level.

The calves that experienced severe (3rd degree) asphyxia demonstrated a decrease of pH by 0.03 units in 4 hours after colostrum giving, but it increased up to the initial level at the end of the experiment. In turn, the BD, MMM, EGH and CMMA indices increased by the end of the experiment by 8.1; 31.1; 36.9 and 8.1% ($p < 0.05$), respectively.

There were no significant differences in immunoglobulin content in calves from various groups before feeding. Changes of the levels of immunoglobulin classes after feeding had the same trend. However, the evidence of these changes was different in the compared groups. Healthy calves and animals after mild asphyxia demonstrated IgG content increase by 5.2 times in 4 hours after feeding, then it increased again by 36.6%. These animals had an increase of IgA level by 10.8-13.5 times and 50.4-54.6% and IgM – by 5.3 times and 36.9% ($p < 0.05$), respectively.

Neonates after asphyxia of second degree of severity had a lower increase of IgG, IgM and IgA content during 4 hours after feeding by 26.8; 35.2 and 38.1% ($p < 0.05$) in comparison with healthy animals (Tab. 4). During the second period of the experiment, absorption of IgG

was by 21.4% more active, but the increase of IgM and IgA did not significantly differ in comparison with indices of the healthy calves.

After severe asphyxia, neonates demonstrated a decrease of immunoglobulin absorption during 4 hours after feeding. IgG decreased by 62.5%, IgM – 61.4% and IgA – 65.3%, and in the final stage they decreased by 9.9; 41 and 38% ($p < 0.05$), respectively.

Table 4.

Immunoglobulin content in blood of calves before (upper line), in 4 (middle line) and 8 (lower line) hours after giving colostrum.

Indices	Healthy	Asphyxia intranatalis (degree of severity)		
		1	2	3
IgG, g/l	0.68±0.005	0.70±0.005	0.68±0.003	0.68±0.005
	3.59±0.017*	3.56±0.007*	2.81±0.003**	1.75±0.003**
	4.90±0.020*	4.90±0.017*	4.4±0.011**	2.93±0.005**
IgM, g/l	0.20±0.004	0.21±0.005	0.20±0.001	0.19±0.003
	1.08±0.003*	1.09±0.005*	0.77±0.001**	0.52±0.001**
	1.47±0.004*	1.48±0.005*	1.15±0.005**	0.75±0.003**
IgA, g/l	0.011±0.0003	0.010±0.0004	0.010±0.0004	0.010±0.0005
	0.130±0.0002*	0.135±0.0007**	0.083±0.0002**	0.051±0.0002**
	0.201±0.0011*	0.203±0.0014**	0.155±0.0010**	0.095±0.0005**

*- $p < 0.05$ significant difference from initial level; ** - $p < 0.05$ significant difference from initial level and indices of healthy calves.

Statistical analysis of experimental data showed that the decrease of immunoglobulin absorption had a significant impact (according to χ^2 -category) on blood pH (rd=0.85), CMMA (rd=0.79) and pO₂ (rd=0.73), medium impact on EGH (rd=0.38), BD (rd=0.40) and pCO₂ (rd=0.32).

Discussion. In healthy calves within 8 hours after feeding, the severity of acidosis, hypoxia, hypercapnia and buffer base deficiency decreases. These changes could be caused by giving colostrum, but probably the main cause was the process of neonates' adaptation to new conditions. According to our data, feeding has become the cause of accumulation of toxic metabolites and increase of adrenoreceptor activity on the membranes of erythrocytes during 4 hours after feeding, but these indices came to norm in the final stage of the experiment. CMMA was an exception. It decreased in 8 hours after feeding, but preserved relatively high level. Probably it is connected with the fact that feeding has a stimulating effect on the state of vegetative nervous system [14].

In calves who have suffered asphyxia within 8 hours after feeding, respiratory-metabolic acidosis, hypoxia, hypercapnia, endogenous intoxication and disturbances of erythrocyte membranes are detected. The degree of manifestation of the above-mentioned diseases and processes depends on severity of the experienced asphyxia.

After giving colostrum, active intestinal immunoglobulin absorption and the increase of their level in blood were observed. This process was the most active during the first hours after feeding. Asphyxia intranatalis does not exclude absorption of immunoglobulins, but the intensity of this process changes. Mild asphyxia did not significantly influence the level of immunoglobulins of calves in comparison with healthy animals. The exception was the tendency to active absorption of IgA during the first hours after feeding. The increase of asphyxia degree was attended by decrease of immunoglobulin absorption. However, the differences in reaction of separate immunoglobulin classes were detected. From 4 to 8 hours, absorption of IgG activated, but absorption of IgM and IgA decreased. Relative tolerance of IgG to negative factors of post-hypoxic period probably was determined by comparatively large quantity of it in colostrum and small size of the molecule. However, during the first hours after feeding, absorption of IgG decreased, as well as absorption of the other classes of immunoglobulins, but the differences became apparent only during the second period of the experiment. That is why, besides the differences in the absorption of separate classes of immunoglobulins, there are compensatory

restoration mechanisms of their absorption processes. These mechanisms also have differences for separate classes of immunoglobulins [15]. Restoration of IgG absorption is the most active.

Results of statistical analysis showed that among blood ABB and gas composition indices pH and hypoxia had the most evident impact on absorption of immunoglobulins. Deficit of bicarbonates and hypercapnia had the least evident impact on absorption of immunoglobulins.

The influence of endogenous intoxication on the processes of absorption of colostrum immunoglobulins has been revealed. More evident inhibition of immunoglobulin absorption was marked at membrane destruction (EGH). In calves after asphyxia there is an increase in adrenoreactivity of erythrocytes (CMMA). It should be noted that the erythrocyte adrenoreceptors do not differ from adrenoreceptors of other cells [16]. This is important for explaining the role of these changes after asphyxia. Since, catecholamines actively influence the consumption of oxygen by the body, organs and cells [17, 18]. Mechanism of this phenomenon can be explained by the fact that intestinal pinocytosis of immunoglobulins is a Ca^{2+} dependent process [19]. Calcium metabolism is regulated by cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), the levels of which depend on the amount of adrenaline (cAMP) and acetylcholine (cGMP) [20].

Conclusion. Neonates have a unique capacity to absorb colostrum immunoglobulins. This capacity changes, but preserves even in neonates in poor condition. Intestinal absorption of immunoglobulins and their penetration into blood decrease in calves that experienced Asphyxia intranatalis.

Inhibition of absorption is the result of pathogenetic factors. Acidosis and hypoxia, as well as membrane destruction, buffer base deficit and hypercapnia, have the strongest impact. Some classes of immunoglobulins have different tolerance to pathogenetic factors of asphyxia. The most rapid absorption recovery is observed in IgG.

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