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EFFECT OF NATIVE AND VITAMINCONTAINING LIPOSOMES ON THE FORMATION OF MATERNAL IMMUNITY OF NEWBORN CALVES

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Due to structural features of cows' placenta, calves are born vulnerable to potentially pathogenic factors. The research presented in this paper was aimed to investigate the level of maternal immunity in the organism of newborns calves using colostrum with its native liposomes and liposomes with vitamins A and E (preparation 'Membranostabil'). Blood serum of newborn calves was used as the material for the study in which total protein and immunoglobulins were tested in the dynamics from birth of animal to 11-day of age.

It was established that use of colostrum with native lecithin liposomes and liposomes containing vitamins A and E 1 time per day increased the level of immunoglobulin in 1.6–2.6 times in the blood serum and prevented indigestion in the newborn calves.

The research results indicated membrane-stabilizing effect of native and rich in vitamins A and E liposomes for plasmolemma of enterocytes of the small intestine in newborn calves.

We suggest that the effect of these preparations is determined by liposomal phospholipids and the ability of vitamins contained in liposomes to maintain a stable structure and viscosity of the plasmic membrane of enterocytes and enhance significantly the activity of proteins of membrane receptors to colostral immunoglobulins.

Keywords: COLOSTRUM, IMMUNOGLOBULIN, MATERNAL IMMUNITY, CALVES, LIPOSOMES, ENTEROCYTES, SMALL INTESTINE

ВПЛИВ НАТИВНИХ І ВІТАМІНОВМІСНИХ ЛІПОСОМ НА ФОРМУВАННЯ КОЛОСТРАЛЬНОГО ІМУНІТЕТУ У НОВОНАРОДЖЕНИХ ТЕЛЯТ

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У зв'язку з особливостями будови плаценти корів телята народжуються беззахисними перед потенційно патогенними факторами. Мета роботи полягала в дослідженні рівня колострального імунітету в організмі новонароджених телят при згодовуванні їм із молозивом нативних ліпосом, а також ліпосом із наявними у них вітамінами А та Е (препарат «Мембраностабіл»). Матеріалом для дослідження була сироватка крові новонароджених телят, в якій у динаміці визначали вміст загального білка та імуноглобулінів, починаючи від народження і до 11-добового віку тварин.

Встановлено, що згодовування новонародженим телятам разом із молозивом 1 раз на добу нативних лецитинових ліпосом і ліпосом, які містять у своєму складі вітаміни А та Е, сприяє підвищенню рівня імуноглобулінів у сироватці крові в 1,6–2,6 разу та запобігає виникненню розладів травлення.

Результати досліджень вказують на мембраностабілізуючу дію нативних і насичених вітамінами А і Е ліпосом на плазмолему ентероцитів тонкого кишечника новонароджених телят.

Висловлено припущення, що дія цих препаратів обумовлена здатністю фосфоліпідів та вітамінів у складі ліпосом підтримувати стабільними структуру і в'язкість плазматичної мембрани ентероцитів та значно підвищувати активність мембранних рецепторних білків до імуноглобулінів молозива.

Ключові слова: МОЛОЗИВО, ІМУНОГЛОБУЛІНИ, КОЛОСТРАЛЬНИЙ ІМУНІТЕТ, ТЕЛЯТА, ЛІПОСОМИ, ЕНТЕРОЦИТИ, ТОНКИЙ КИШЕЧНИК

ВЛИЯНИЕ НАТИВНЫХ И ВИТАМИНСОДЕРЖАЩИХ ЛИПОСОМ НА ФОРМИРОВАНИЕ КОЛОСТРАЛЬНОГО ИММУНИТЕТА У НОВОРОЖДЕННЫХ ТЕЛЯТ

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В связи с особенностями строения плаценты коров телята рождаются беззащитными перед потенциально патогенными факторами. Цель работы заключалась в исследовании уровня колострального иммунитета в организме новорожденных телят при скармливании им с молозивом нативных липосом, а также липосом с заключенными в них витаминами А и Е (препарат «Мембраностабил»). Материалом для исследования служила сыворотка крови новорожденных телят, в которой в динамике определяли количество общего белка и иммуноглобулинов, начиная от рождения и к 11-суточному возрасту.

Установлено, что применение новорожденным телятам 1 раз в сутки вместе с молозивом нативных лецитиновых липосом и липосом, которые содержали в своем составе витамины А и Е, способствует повышению уровня иммуноглобулинов в сыворотке крови в 1,6–2,6 раза и предотвращает появление расстройств пищеварения.

Результаты исследований указывают на мембраностабилизирующее действие нативных и насыщенных витаминами А и Е липосом по отношению к плазмолемме энтероцитов тонкого кишечника новорожденных телят.

Высказано предположение, что действие этих препаратов обусловлена способностью фосфолипидов липосом и заключенных в них витаминов поддерживать стабильными структуру и вязкость плазматической мембраны энтероцитов и значительно повышать активность размещенных на ней иммунорецепторных белков к иммуноглобулинам молозива.

Ключевые слова: МОЛОЗИВО, ИММУНОГЛОБУЛИНЫ, КОЛОСТРАЛЬНЫЙ ИММУНИТЕТ, ТЕЛЯТА, ЛИПОСОМЫ, ЭНТЕРОЦИТЫ, ТОНКИЙ КИШЕЧНИК

Due to the peculiarity of the structure of a cows' placenta, which prevents the passage of antibodies from the maternal organism to foetal one, newborn calves are exposed to potentially pathogenic factors. Therefore, humoral immune defence plays the main role in protection of calves during the early postnatal period because of the formation of a sufficient level of passive (maternal, colostral) immunity [1–4].

The level of maternal immunity depends on the amount of protein of γ-globulin fraction coming from colostrum of cow to the organism of newborn calf during the early days of its life [5, 6]. Further penetration of antibodies in permanent form through the mucous membrane of the small intestine of the calf is much slower, and stops completely after 24–36 hours [2, 7–9].

The formation of maternal immunity in newborn calves is affected by many different factors such as the time of the administration of the colostrum after birth, temperature of colostrum, frequency and amount of feedings, content of immunoglobulins in the colostrum and the amount that absorbed proteins through the mucous membrane of the small intestine [2, 10, 11].

One of the important factors determining the formation of passive immunity in newborn calves is the ability of enterocytes in the small intestine to absorb effectively immunoglobulins from colostrum. This immunity is determined by structural and functional features of plasmolemma of enterocytes in the small intestine of the calf during neonatal period, which depends on its lipid and protein composition, density, presence of membrane receptors for polypeptide antibodies, active transport ADPase and other factors [3, 4, 12].

Based on the above, the aim of this study was to investigate the level of maternal immunity in the organism of newborn calves using colostrum with native liposomes based on soy lecithin, and liposomes including vitamins A

and E, and to examine the relationship between the level of maternal immunity and development digestive disorders in these animals.

Materials and methods

The study was conducted on newborn calves of black-motley breed in the period from birth to 11 days of age at an experimental farm «Velykosnitynske named after A. Muzychenko» of NULES of Ukraine.

Three groups (one control and two experimental) of calves were established. There were five animals in each group. Calves of all groups were fed with two liters of colostrum after birth, and then — 1.5 litres every 4 hours at the first day and every 6 hours at the second and the third days of life. At the fourth day of the calves' life they were fed three times. Calves of the first experimental group also got native liposomes in the form of macrocapsule (diameter of liposome was 46.5 nm) in an amount of 5 ml during the morning feeding with colostrum. Calves of the second experimental group also got the same amount of liposomes containing vitamins A and E. The content of vitamins in liposomes was: A — 0,04 g, E — 15 mg during the morning feeding with colostrum.

Blood samples were taken from the jugular vein of calves and stored in vacuum tubes, from the birth of animals until the administration of the colostrum and after 6, 24, 72 hours and at the 7th and 11th day of life. Blood serum was obtained by separation from the formed elements in an incubator at a temperature 37° C within 1 hour followed by centrifugation at 3000 rpm for 15 minutes. The content of total protein in the blood serum was determined by the biuret reagent and protein fractions — by electrophoresis in 7.5 % polyacrylamide gel according to the method by Laemmli [13]. Protein content of immunoglobulin fraction (g/l) in the blood serum of calves was determined by calculation counting the number of albumin, from the index of total protein).

Biochemical parameters of blood plasma were determined by standard methods using biochemical analyzer «Lab Line 010», № K 05-9033, (firm LabLine Diagnostics, representative «West Medica productions and Handels GmbH» Approval Certificate of Measuring Instruments EN ISO 9001:2000 Cert No: 2010083000258), Austria (certificate of calibration device number 2344 / T on December 6, 2012). Reagents firm SpaynLab: diagnostic reagents for biochemical studies U24.4-36035842-001 TU 200 (certificate of registration № 9094/2009, valid until December 3, 2014) and sets of reagents for clinical biochemistry (certificate of registration № 9670/2010, valid until August 12, 2015) (producer High Thechnology, Inc., USA) were used in conducting biochemical studies.

Results and discussion

Before feeding newborn calves with colostrum, the content of total protein in the blood serum was 43.8±1.5 g/l (Fig. 1), which is consistent with data obtained in other studies [2, 12, 14, 15].

After receiving colostrum, total protein in the blood serum increased significantly due to active transport processes in the small intestine of animals during neonatal period.

During the research we had set a trend to increased concentration of total protein in the blood serum of newborn calves in the control group. Moreover, its concentration in the blood serum to the 3rd day of life of calves in the control group increased in 1.13 times compared with the beginning of the experiment and remained relatively stable until the 11th day of age.

At the same time, in the blood serum of newborn calves of first experimental group which got colostrum with liposomes as macrocapsule we found a significant increase of total protein concentration in 1.26 times at the 6th hour of their life; and these data were significantly higher (in 1.26–1.31 times, $P<0.05$) to 11 days of age of the animals compared to calves of the control group (see *Fig. 1*).

During the experiment in the blood serum of calves of the second experimental group that received colostrum with liposomes containing vitamins A and E it was also found higher concentration of total protein (in 1.10–1.15 times) than in calves of the control group.

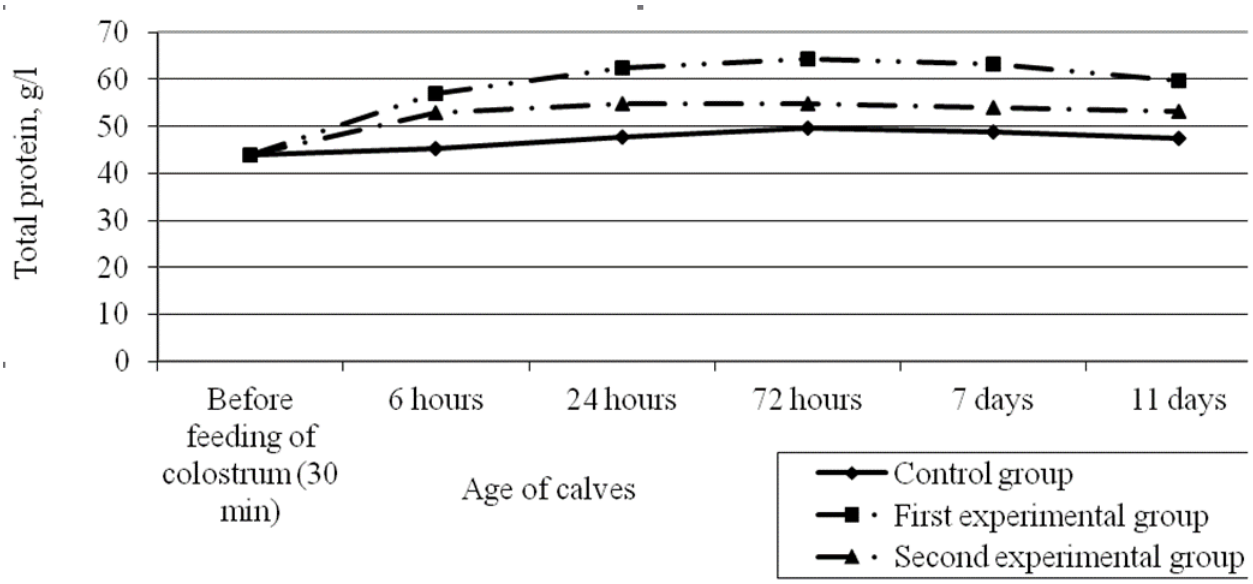

Fig 1. The content of total protein in the blood serum of calves, M±m, n=5.

However, this rate was significantly lower in 1.12–1.20 times (P<0.05) compared to calves of the first experimental group throughout the study period (see *Fig. 1*).

As a result of the colostrum feeding protein content of minor immunoglobulin fraction in the blood serum of newborn calves was 0.31±0.06 g/l (see *Fig. 2*). This confirms that only about 5 % of immunoglobulins can pass the trans-placental barrier from the cow-mother to foetus, and the others must enter the organism of newborn calf with the colostrum.

After feeding calves with colostrum, the immunoglobulins concentration of all groups in their blood serum increased rapidly.

Thus, after the first colostrum administration, concentration of immunoglobulins in the blood serum of newborn calves in the control group increased to 11.0±0.06 g/l, and subsequently it significantly increased in 1.19 and 1.35 times to the 24th and 72nd hours of life, respectively. Then in the blood serum of calves in the control group a significant decrease of Ig in 1.17 (P<0.01) and 1.36 times (P<0.001) at the 7th and 11th day was observed respectively, compared with these parameters at the 72-nd hour of the animals' life (see *Fig. 2*).

After applying native liposomes with colostrum to the newborn calves of first experimental group the protein content of immuno-

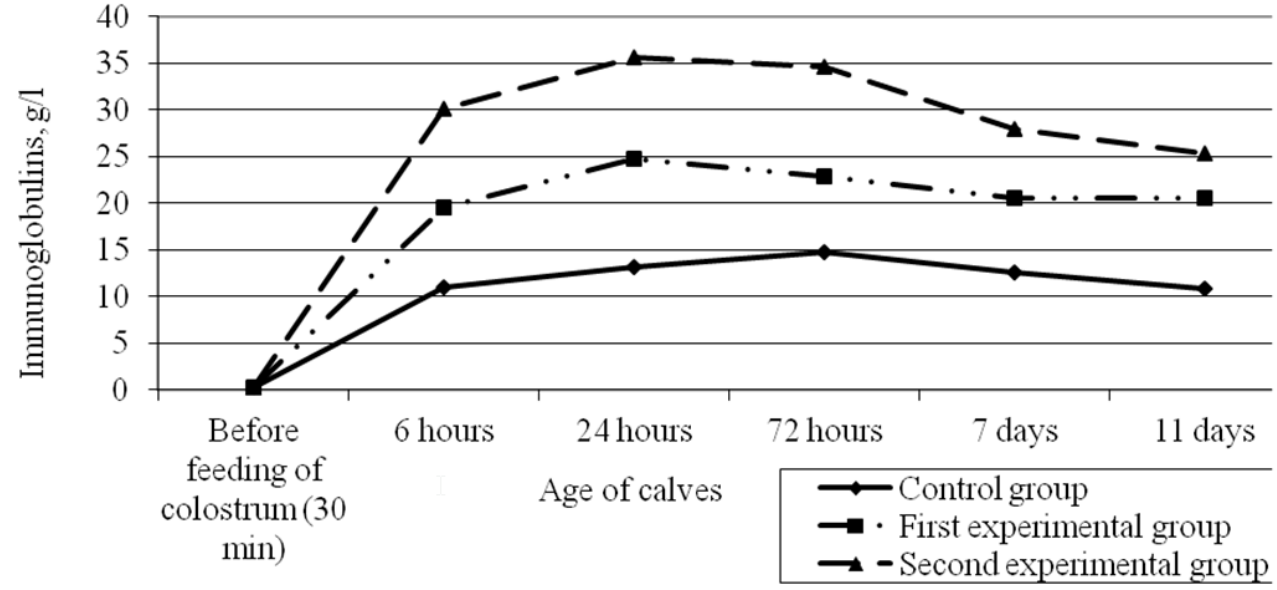

Fig 2. The content of immunoglobulins in the blood serum of calves, M±m, n=5.

globulin fractions in the blood serum increased to 19.5±1.5 g/l at the 6th hour of age, which is in 1.77 times higher (P<0.001) compared to calves of the control group. At the first day of life calves of the first experimental group showed an increase in the concentration of immunoglobulins in their blood serum as much as 1.27 times which was already 24.8±0.23 g/l. Further, there was a tendency to a slight reduction in serum Ig concentration of calves, which stabilized to 7-day old animals and did not change until the 11th day of life and was 20.5±0.11 g/l. Despite the declining trend in the protein level of immunoglobulin fractions in the blood serum of calves of the first experimental group from the first hours to 11th day of life was in 1.55–1.90 times higher (P<0.001) compared to calves of the control group (see *Fig. 2*).

Application of liposomes with the inclusion of vitamins A and E in colostrum given to the calves of the second experimental group showed increase of the protein content of immunoglobulin fractions in the serum of blood to the 6th hour of age to 30.15±1.24 g/l, and the 24th hour — to 35.6±0.29 g/l. This figure has remained almost unchanged up to 3 day's of calves age of the second experimental group (34.6±0.56 g/l). In the future, the concentration of immunoglobulins in the blood serum of these animals gradually decreased to 11 days of age and was 25.4±0.69 g/l. Note, however, that the index in the second experimental group of calves was in 1.87 (P<0.001) and 1.24 (P<0.05) times significantly higher than in calves of the control group and the first experimental group, respectively.

It should be brought to notice the level of immunoglobulins in the blood serum of newborn calves during the first 3 days of age are critical for the development of indigestion. Thus, the available data show [12, 14–16] that if the content of immunoglobulins in the serum of calves in this period is lower than 10–12 g/l there is an increased risk of digestive disorders. Therefore, increasing the level of immunoglobulins in the blood serum of calves during the neonatal period and the formation of maternal immunity and maintenance of the appropriate level of immune proteins in the blood of animals prior to the production of the body's own antibodies would avoid development of such disorders, and later — other potentially possible pathologies.

In view of the above, tools of reparative therapy such as a native lecithin liposomes and liposomes containing supplements of vitamins A and E, as well as our results, indicate a significantly higher (in 1.6–2.6 times) level of colostral immunity in these animals compared with the control group of calves which were clinically evaluated in terms of acute indigestion with symptoms of diarrhea.

Thus, throughout the experiment two calves of the control group from the second day of age and another one calf of the same group on the third day of age noted digestion with symptoms of diarrhea accompanied by dehydration. Animals were depressed, refused to feed, and lacked proper sucking reflex. Calves lying in the thoracic position were periodically inspected e.g. stomach, stool was watery with different shades of yellow and a stinking smell. For the treatment of these animals we had to use rehydration therapy, styptics, ambient preparations and other tools up to 6–7 days or more prior to their recovery.

At the same time, in the calves of the first and second experimental groups there were no cases of digestive disorders during the experiment. Animals were active, had a good appetite, showed good sucking reflex, hair was shiny and supple, stool was formed, dark yellow. In the second experimental group calves were more active than calves of the first experimental group.

Conclusions

Thus, our results on the content of total protein and immunoglobulins in the serum of newborn calves of research groups, as well as data on the clinical condition of the animals showed efficient performance of native liposomes rich in vitamins A and E on liposome formation and the level of maternal immunity in these animals.

The experimental results indicate the membrane-stabilizing effect of liposomal macrocapsule medications in native form and by in-

cluding vitamins A and E in relation to the enterocytes of the small intestine of newborn calves. The effect of these medications is due to the property of the phospholipids that make up their composition to maintain stable structure and viscosity of plasmolemma of enterocytes. This, in turn, determines the activity of immunoreceptor proteins of enterocytes' plasmolemma to immunoglobulin colostrum, contributes a sufficient level of maternal immunity and prevents digestive disorders in newborn calves.

At the same time the results of our research revealed that macrocapsular preparation of liposoms including vitamins A and E, compared to native liposomes, showed more pronounced effect on the formation of maternal immunity in newborn calves. It can be explained by the vitamin A abillity to affect the microviscosity of cell membrane lipids through regulation the ratio of saturated and unsaturated fatty acids in the lipid layer. On the other hand, vitamin E stabilizes cell membranes by its interaction with proteins, phospholipids and free fatty acids, and provides them with antioxidant protection. This is particularly important in a period of intense transport of macromolecular compounds (protein immune colostrum) in the small intestine of animals during newborn period [12, 17].

Perspectives of future research. However, statements regarding the impact of vitamin A and E for the formation of maternal immunity in newborn calves need further investigations. The aim of these researches is to establish qualitative and quantitative composition of lipids and proteins of enterocytes' plasmolemma in the small intestine of animals. It needs providing of clinical experiments.

Therefore, further research will focus on establishing the structure and biochemical function of enterocytes' plasmolemma. Also investigations will study the processes of transport of certain classes of immunoglobulins (IgG) in the small intestine of newborn calves under the influence of native liposomes and preparation «Membranostabil».

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