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Functional Activity Of Thrombocytes In Newborn Calves.

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ABSTRACT

In newborn calves of black and mottled breed of optimal physiological status, with a normal amount of platelets in the bloodstream, there is no reliable dynamics of platelet aggregation in response to adenosine diphosphate, collagen, thrombin, ristomycin, adrenaline, and combinations of ADP and adrenaline induction inhibitors, ADP and collagen and collagen and adrenaline. The level of discocytes in their blood on the 1st-2nd day was 77.2%, not changing reliably until the end of the phase of the newborn. The number of disco-echinocytes, spherocytes, sphero-echinocytes and bipolar forms of platelets in the blood stream remained stably low. Important mechanisms that ensure the stability of aggregation activity of platelets in calves in the phase of newborns can be considered the preservation during a mammal nutrition of low intensity of metabolism in them of arachidonic acid and a low content of adenosine triphosphate and adenosine diphosphate, as well as actin and myosin.

Keywords: calfs, a phase newborn, early ontogenesis, thrombocytes, lipid peroxidation.

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INTRODUCTION

The solution of the main social problems of our time is not conceivable without the provision of sufficient food for society [1,2,3]. An important source of it is considered animal husbandry [4]. Its intensification, required at the present time, is possible with the firm support of practice for new physiological knowledge of cattle. It becomes clear that systems integrating the organism are extremely important in the rate of development of the productive qualities of calves [5,6]. Blood is very important [7,8]. Its rheological properties are especially important for the metabolism of tissues [9,10]. Platelets entering the hemostatic system play a major role in the successful movement of blood through the capillaries [11,12,13].

In this connection, one of the most important components of the homeostasis of a growing organism is the effective functioning of the system of platelet hemostasis, which largely determines the fluid properties of the blood, on which the development of the organism in the phase of newborn development as the basis of all subsequent early ontogenesis largely depends [14]. The level of functional activity of platelet hemostasis is largely related to the anatomical formation of the body's structures, the development of their functional activity due to the maximum realization of the body's genetic program [15,16]. In connection with this, it is important to study the aspects of platelet activity in calves, which are an important element in maintaining the homeostasis of the internal environment in general and the blood system in particular in the phase of newborns [4].

The purpose of this study is to study the physiological features of platelets in healthy calves in the phase of newborns.

MATERIALS AND METHODS

Research was conducted in strict accordance with ethical principles established by the European Convent on protection of the vertebrata used for experimental and other scientific purposes (adopted in Strasbourg March 18, 1986, and confirmed in Strasbourg June 15, 2006) and approved by the local ethic committee of Russian State Social University (Record №12 dated December 3, 2015).

The studies were carried out on 29 healthy calves of black-motley and Simmental breeds, which were examined 5 times in the phase of newborn: on 1-2 days, 3-4, 5-6, 7-8 and on 9-10 days. Platelet aggregation was assessed by visual micromethod using ADP ($0.5x10^{-4}$ M), collagen (1:2 dilution of the main suspension), thrombin (0.125 U/ml), ristomycin (0.8 mg/ml) as inductors, epinephrine ($5.0x10^{-6}$ M), as well as combinations of ADP+adrenaline, ADP+collagen and collagen+epinephrine in similar concentrations with a standardized platelet count in the plasma of $200x10^{9}$ platelets tested.

For an indirect evaluation of the metabolism of endogenous arachidonic acid in platelets, as well as the activity of cyclooxygenase and thromboxane synthetase in them, three transfer samples were used with registration of platelet aggregation on a photoelectrocolorimeter.

The content of platelets ATP and ADP, the amount of their secretion under the influence of collagen and the dynamics of the protein composition of the cytoskeleton of blood plates (actin and myosin) were determined with the activation and aggregation of platelets under the action of ADP and thrombin. Intravascular activity of thrombocytes was assessed using a phase contrast microscope. The results of the study were processed using the Student's test.

RESULTS OF THE STUDY

The study of platelet activity in healthy newborn calves revealed a lack of reliable dynamics of the functions of the blood platelets while maintaining the content of platelet count in the bloodstream of animals within normal limits.

In calves on the 1st and 2nd day of life, the time of development of platelet aggregation under the influence of collagen was 31.4±0.14 seconds, and on the average for the phase of newborns - 31.4±0.08 s. A similar dynamics of platelet aggregation was observed under the influence of ADP (average 40.2±0.08 s) and ristomycin (average 48.0±0.12 s). Several thrombin (an average of 53.8±0.07 s) and adrenaline (an average of

98.2±0.06 s) thrombocyte aggregation developed somewhat slowly, and in the absence of their reliable dynamics during the phase of mammary nutrition. Absence of this dynamics was also revealed against the background of their combinations: for ADP+adrenaline 38.0±0.09 s, for ADP+collagen - 27.9±0.06 s, for adrenaline + collagen 30.8±0.07 s, that is, the in vitro established time of development of platelet aggregation by the time of its occurrence in healthy animals in vivo under real conditions of blood flow in the phase of newborns is closer to that.

The level of discocytes in the blood of calves remained stably high throughout the entire phase of the newborn (Table). The number of disco-echinocytes, spherocytes, sphero-echinocytes and bipolar forms of platelets also remained stable during the first 10 days of life. As a result, the total number of active forms of platelets did not change significantly [17]. The content of freely circulating small and large aggregates of platelets remained practically at the same level during the entire first phase of ontogenesis, as well as the number of platelets involved in the aggregation process.

Table. Intravascular activity of thrombocytes in calves in the phase of newborn (n=29, M±n)

Registered Parameters	1-2 day of life	3-4 day of life	5-6 day of life	7-8 day of life	9-10 day of life	Mean values
Discolets,%	77.2±0.08	78.0±0.12	78.1±0.16	77.5±0.07	77.8±0.15	77.7±0.11
Disco-echinocytes,%	14.2±0.12	13.9±0.14	13.5±0.13	14.6±0.18	13.7±0.09	13.9±0.13
Spherocytes,%	5.0±0.05	4.4±0.03	4.6±0.07	4.4±0.06	4.5±0.08	4.7±0.06
Sphero-echinocytes,%	2.8±0.06	2.5±0.03	2.7±0.05	2.5±0.04	2.9±0.08	2.7±0.05
Bipolar forms, %	0.8±0.02	1.2±0.03	1.1±0.04	1.0±0.03	1.1±0.02	1.0±0.03
Sum of active forms,%	22.8±0.06	22.0±0.11	21.9±0.14	22.5±0.18	22.2±0.10	22.3±0.11
The number of platelets in the aggregates,%	4.6±0.05	4.9±0.09	4.8±0.07	5.2±0.10	5.1±0.07	4.9±0.07
The number of small aggregates - 2-3 platelets per 100 freely lying platelets	3.3±0.08	3.4±0.07	3.6±0.03	3.5±0.06	3.7±0.09	3.5±0.06
The number of medium and large aggregates - 4 or more platelets per 100 freely lying platelets	0.13±0.07	0.14±0.03	0.16±0.06	0.13±0.05	0.14±0.08	0.14±0.07

One of the mechanisms that ensure the stability of platelet aggregation activity in calves in the phase of newborns can be considered the preservation during low-dose feeding of low intensity of thromboxane synthesis in platelets, which could be indirectly judged by platelet aggregation in a simple transfer sample [18] (average per phase 30.2±0.06%). Persistence of the level of arachidonic acid metabolism in the blood plates of healthy newborn calves was possible due to the lack of reliable dynamics of both enzymes of its transformation in platelets - cyclooxygenase and thromboxane synthetase. Restoration of platelet aggregation in a collagen-aspirin test, indirectly assessing cyclooxygenase activity in platelets, was 78.4±0.05%. Restoration of platelet aggregation in the collagen-imidazole sample, which allows to indirectly determine the state of thromboxane synthetase in blood plates, also did not significantly change during the neonatal phase and amounted to an average of 39.4±0.06%.

The initially low content of ATP and ADP in calf platelets remained stable for all 10 days (5.48±0.10 and 3.27±0.09 μmol / 109 thrombocytes, respectively). The level of their secretion from platelets did not change and was 30.3±0.09 and 40.2±0.11%.

The content of actin and myosin on day 1-2 was 26.7±0.11 and 12.1±0.16% of the total protein in the platelet, and on average 28.1±0.10% and 13.0±0.11% for the phase eleven. The intensity of additional actin formation on the background of activation by a strong or weak inducer and during subsequent aggregation under their influence in calves also did not change reliably during the entire phase of the newborn.

DISCUSSION

The revealed stability of the activity of blood platelets in healthy newborn calves, ensured by the constancy of their individual functional mechanisms, largely determines the necessary rheological properties of blood in subsequent phases of ontogenesis. In this regard, the analysis of the level of activity of platelet hemostasis and the fine mechanisms of its implementation in healthy calves in the phase of colostrum nutrition is relevant.

In healthy newborn calves, the adhesive capacity of the blood platelets did not change due to the constant concentration of the von Willebrand factor (WF) - the coagulant of platelet adhesion [19,20] and/or the lack of dynamics of the number of receptors for it (GPI) on the surface of blood plates [21,22]. The stability of the level of WF in newborn calves is determined on the basis of the lack of dynamics of platelet aggregation with ristomycin, which by the ability to influence platelets is identical to the subendothelial structures of the vessels [23,24]. It is known that WF is connected by one end of the molecule with collagen and the other with a platelet through the receptor glycoprotein 1b, forming the "adhesion axis": collagen- WF-GPIv, which suggests the stability in calves in the neonatal phase of the number of these receptors on platelet membranes [25, 26]. The invariance of platelet aggregation with the rest of the inducers was determined by the constancy of the calves in the first 10 days of life of the number of receptors to them on the membranes of the blood plates [27,28].

The investigation of the effect of two aggregation inducers in combination with the process of platelet aggregation in newborn animals speaks of their mutual potential influence under physiological conditions [29,30]. Registration of platelet aggregation with a combination of its inducers simulates real blood flow conditions in growing calves, allowing one to evaluate platelet aggregation when stimulated by various pathways, which is typical for normal blood flow conditions [31,32]. The level of discocytes in the blood was low and did not change reliably during the milk supply [33], which led to the stability of the sum of the active forms of platelets and the level of platelet aggregates in the blood [34,35]. At the heart of this was the non-expressed blood flow in the bloodstream, with the formation of processes with a transition from discocyte to disco-echinocyte, then to spherocyte and sphero-echinocyte [36,37]. This resulted in low hemostatic activity of platelets with ineffective adhesion and in vivo aggregation associated with a slight expression of fibrinogen receptors (GP IIb-IIIa) on their membranes and stimulation of the catalytic properties of plasma membrane phospholipids, providing a low level of generation of factor Xa and thrombin [38,39].

CONCLUSION

The revealed regularities of platelet activity in healthy calves in the newborn phase determine the optimal level of intravascular activity of platelets and microcirculation in tissues, which is adequate on one hand to their genetic program, on the other hand, to the influence of the external environment, and can be considered a specific adaptive response to the onset of extrauterine existence [40].

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