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Functional Parameters Of Platelets In Calves Of Yaroslavl Breed In The First 10 Days Of Life.

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ABSTRACT

The functional state of platelets in cattle can vary depending on many factors external and internal environment. Of great interest is the status of their activity at the beginning of ontogenesis, which may vary in different species of animals. The aim of this work is to find out the activity of platelets in newborn calves in the Yaroslavl breed. The study was conducted on 34 of the Yaroslavl breed calves obtained from healthy cows after 2-3 of pregnancy. All calves were examined for the planning of 1-2, 3-4, 5-6, 7-8 and 9-10 day of their lives. The applied biochemical, hematological and statistical methods of research. The examined calves during the neonatal phase, a tendency to increased activity of platelet aggregation in response to all tested inducers. The number of discoid platelets in the blood of calves of the Yaroslavl breed experienced a tendency to decrease during the observation period. The number of victims of platelet activation during the observation they have increased by 10.4%. The number of circulating blood of small and medium and large aggregates of platelets also had their rising trend during the phase of neonatal. These changes are largely provided in calves Yaroslavl breed a tendency to increase synthesis of thromboxane in platelets. This occurred as a result of the strengthening in them of cyclooxygenase and thromboxane synthetase. A large role was played by increase of a level of content in platelets of adenosine phosphates and a tendency to increase their secretion. The quantitative content of actin and myosin in intact platelets calves in the Yaroslavl breed was increased in the phase of neonatal 6.7% and 13.8%, respectively. While they have a marked tendency to increase synthesis of additional actin and myosin on the background of platelet aggregation by 6.7% and 10.4%, respectively. The data obtained suggest that newborn calves in the Yaroslavl breed is characterized by low activity of aggregation and secretion of platelets. This ensures that they have good conditions for the process of microcirculation. Small intravascular platelet activity in those calves creates the necessary conditions for metabolism in the tissues, promoting their growth and development.

Keywords: calves, newborn Yaroslavl breed, platelets, aggregation, secretion.

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INTRODUCTION

The physiological features of the hemostasis system to a large extent determine the processes of hemocirculation [1,2]. Great importance in this belongs to platelets. Their hemostatic activity strongly determines the success of the microcirculation [3,4] in any living organisms [5,6]. In previous studies, it was noted that platelet activity may change during active growth [7] with aging [8,9] against the background of the formation of dysfunctions [10,11], the formation of obvious pathology [12], the appearance of vasopathy [13,14] and conditions of various therapeutic effects on the body [15,16,17]. However, many aspects of the functioning of platelet hemostasis in cattle are not studied. It was possible to find only individual works on the assessment of the state of platelets in these productive animals, taking into account their genetic features at certain stages of their ontogenesis [18]. This scattered information does not allow for a holistic view of this issue and creates a need for continuing systematic research. The importance of these studies is provided by the high significance of platelet activity for capillary blood flow. It is recognized that microcirculation determines the intensity of growth and development of animals and its individual organs, realizing their productive potential [19]. Considering the presence of genetic differences between cattle breeds and the high physiological significance of platelet activity for the realization of productive potential, it was very important to assess the functional characteristics of platelets in calves highly productive in terms of the milkiness of the Yaroslavl breed at the start of the realization of their functional features in ontogenesis.

The goal - to find out the activity of platelets in newborns of te¬lyat Yaroslavl breed.

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 All-Russian SII of Physiology, Biochemistry and Animals' feeding (Record №11, dated December 4, 2015).

The study was performed on 34 calves of the Yaroslavl breed, obtained from healthy cows, 2-3 pregnancies. The calves were examined and examined during the neonatal phase 5 times: 1-2, 3-4, 5-6, 7-8 and 9-10 days of life.

The indirect assessment of the intensity of thromboxane synthesis in platelets and the indirect evaluation of the enzymatic activity of cyclooxygenase and thromboxane synthetase in this process were carried out in three transfer samples using a photoelectrocolorimeter [20]. In platelets, the amount of adenosine triphosphate (ATP) and adenosine diphosphate (ADP), the activity of their secretion when collagen appears in the medium, and the level of actin and myosin in the protein cytoskeleton of intact and activated platelets under the influence of ADP [20].

The time of platelet aggregation (AP) was evaluated using a visual micromethod [21], using as inducers ADP (0.5×10^{-4} M), collagen (dilution 1: 2 of the main suspension), adrenaline (5.0×10^{-6} M), thrombin (0.125 units/ml) and ristomycin (0.8 mg/ml) in plasma standardized by the number of platelets to 200×10^9 platelets. The level of intravascular platelet activity was determined by phase-contrast microscopy [22].

Statistical processing of received information was made with the help of a programme packet “Statistics for Windows v. 6.0”, “MicrosoftExcel”. Differences in data were considered reliable in case $p<0.05$.

RESULTS

In newborn calves of the Yaroslavl breed, a tendency was found to enhance the initially low functional activity of platelets. Thus, at the 1-2 days of life, taken from the observation of calves, AP appeared in response to collagen for 32.3±0.14 s, subsequently accelerating by 9-10 days of their life to 30.5±0.14 s. A similar tendency to accelerate the AP process was detected in response to ADP and ristomycin up to 36.6±0.13s and 46.6±0.15s, respectively. Moreover, AP with thrombin and adrenaline also showed a tendency to reduce the time of its occurrence to 49.6±0.13 s and 92.1±0.14 s.

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Table: Platelet indicators in newborn calves of Yaroslavl breed

Registered indicators	Calves of Yaroslavl breed, n=34, M±m				
	1-2 day	3-4 day	5-6 day	7-8 day	9-10 day
ATP content in platelets prior to the start of secretion, µmol/10^9 platelets	5.52±0.014	5.54±0.010	5.58±0.016	5.62±0.013	5.67±0.019
The content of ADP in platelets before the start of secretion, µmol/10^9 platelets	3.31±0.005	3.32±0.003	3.39±0.007	3.43±0.012	3.46±0.009
ATP secretionlevel, %	32.3±0.10	33.5±0.16	33.9±0.08	34.5±0.09	34.9±0.14
ADP secretionlevel,%	43.4±0.12	43.6±0.13	43.9±0.16	44.6±0.14	45.6±0.20
The degree of recovery of AP during the collagen-aspirin test, %	82.3±0.07	82.5±0.05	82.6±0.06	83.0±0.10	83.6±0.13
The degree of recovery of AP during the collagen-imidazole test, %	42.4±0.02	42.5±0.06	42.9±0.05	43.5±0.10	44.6±0.12 p<0.05
AP activity in simple transfer assay, %	31.8±0.06	31.9±0.12	32.0±0.04	33.2±0.08	34.2±0.09 p<0.05
Actin content in inactive platelets,% to total protein in platelets	31.4±0.10	31.5±0.08	31.9±0.11	32.6±0.16	33.5±0.10 p<0.05
The content of actin in platelets with ADP-aggregation,% of total protein in platelets	39.0±0.15	39.2±0.14	39.7±0.08	40.5±0.05	41.3±0.12 p<0.05
The content of myosin in inactive platelets,% of total protein in platelets	14.5±0.13	14.7±0.14	15.0±0.12	15.6±0.16	16.5±0.15 p<0.05
The content of myosin in platelets with ADP-aggregation,% of total protein in platelets	28.9±0.20	29.1±0.17	29.6±0.14	30.5±0.17	31.9±0.19 p<0.05
Time of onset of AP with ADP, s	38.1±0.12	38.0±0.16	37.8±0.07	37.1±0.05	36.6±0.13
Time of onset of AP with collagen, s	32.3±0.14	32.2±0.12	31.7±0.15	31.2±0.11	30.5±0.14
Time of onset of AP with thrombin, s	50.1±0.10	55.0±0.08	49.8±0.07	49.7±0.10	49.6±0.13
Time of onset of AP with ristomycin, s	48.2±0.15	48.1±0.17	47.6±0.14	47.2±0.20	46.6±0.15
TimeofonsetofAPwithadrenaline, s	95.2±0.22	94.7±0.16	93.5±0.21	93.0±0.18	92.1±0.14
Platelet-plateletcount, %	75.2±0.19	74.8±0.16	74.0±0.18	73.3±0.14	72.6±0.16
Sum of active forms of platelets, %	24.8±0.12	24.2±0.16	26.0±0.14	26.7±0.18 p<0.05	27.4±0.17 p<0.05

The number of small platelet aggregates per 100 free platelets	3.9±0.08	4.1±0.04	4.4±0.02 p<0.05	4.8±0.03 p<0.01	5.3±0.05
The number of medium and large platelet aggregates per 100 free platelets	0.15±0,020	0.16±0.018	0.17±0.014 p<0.05	0.18±0.022	0.19±0.027

Note: p - the reliability of the dynamics of indicators in relation to 1-2 daily age.

The level of platelet-discocytes in the blood of the examined calves during the neonatal phase experienced a tendency to decrease. During the observation of the calves, the sum of the active forms of platelets in their blood increased by 10.5%. The number of platelet aggregates of various sizes circulating in their blood also increased significantly in their first 10 days of life.

An important mechanism that forms a tendency to increase, AP in newborn calves of the Yaroslavl breed, can be considered to be a tendency to increase in their platelets of thromboxane synthesis. This was indirectly judged by the increase in AP in a simple transfer test, which constitutes 34.2±0.09% for 9-10 days of life. At the basis of this, in the observed calves there was a tendency to an increase in the activity of cyclooxygenase and thromboxane synthetase in their platelets. This was indicated by an increase in the degree of AP recovery in a collagen-aspirin sample indirectly evaluating cyclooxygenase activity in platelets (by the end of the observation, 83.6±0.13%). The level of AP recovery in a collagen-imidazole sample indirectly evaluating the activity of thromboxane synthetase in the platelets of the calves also increased and was 44.6±0.12% on day 9-10.

The initially low content in the platelets of calves ATP and ADP tended to increase, reaching by 9-10 days of life 5.67±0.019 and 3.46±0.009 µmol/10^9 platelets. Under these conditions, the activity of their secretion from platelets during the first 10 days of life experienced a tendency to increase to 34.9±0.14% and 45.6±0.20%.

The content of actin and myosin on the 1-2 day in inactive platelet calves was 31.4±0.1% and 14.5±0.13% of the total protein in the platelet, and by the end of the observation 33.5±0.10% and 16.5±0.15% of the total protein in the platelet. In the process of observation in calves, a tendency was found to increase the formation of actin and myosin under the conditions of platelet aggregation.

DISCUSSION

Long-term studies have allowed to recognize the high biological significance of hematological studies in humans and animals, as they can help uncover many aspects of the functioning of mechanisms for maintaining homeostasis in mammals [23,24]. Despite the great importance for the functioning of the body of young, highly productive breeds of cattle platelet activity, taking into account the pedigree, remains poorly investigated. This was the reason for conducting the present research on the Yaroslavl breed.

The assessment of these calves AP in response to collagen and ristomycin was initially low, indicating an unexpressed ability to adhere their platelets during the newborn, but experiencing a tendency to increase. Obviously, this was based on two mechanisms [25]. It was possible to speak about the first mechanism, relying on the found tendency to accelerate the aggregation of blood platelets in response to collagen. This may be due to a gradual increase in the number of collagen receptors, glycoproteins Ia - IIa and VI, on the surface of the platelets of calves during the neonatal phase. As a second mechanism for enhancing the adhesion of platelets in calves of the Yaroslavl breed, an increase in their surface of the von Willebrand factor (GPIb) receptors on their surface while simultaneously increasing this substance in their blood. This was indicated by the acceleration of antibodies with ristomycitis detected in the examined calves [26].

Identified in newborn calves of Yaroslavl breed, the tendency to accelerate platelet aggregation, apparently, provides a high protection of their body from blood loss. A slight acceleration of their antibodies in response to strong aggregation inducers (collagen and thrombin) seems to be associated with a slight increase in their receptors on platelets with a slight activation of phospholipase C and the phosphoinositol

pathway and mild stimulation of phosphorylation of proteins of the contractile system [27]. The tendency to an increase in the formation of inositol triphosphate in platelets of calves of the Yaroslavl breed was apparently provided by a slight increase in the yield of Ca^{2+} from its depot, causing an increase in their self-assembly process and a reduction in actomyosin [28].

In response to weak aggregation inducers (ADP and adrenaline) in calves of the Yaroslavl breed, the process of platelet aggregation tended to accelerate. This was apparently provided by an increase in the receptor density on the platelet surface, physiological enhancement of the expression of fibrinogen receptors (GPIIb-IIIa) and some increase in the activity of phospholipase A_2 in them. The latter mechanism allowed a certain amount of arachidonic acid to emerge from membrane phospholipids, which contributed to a tendency towards enhanced synthesis of thromboxane A_2 [29]. In addition, the tendency to increase in functional capabilities of cyclooxygenase and thromboxane synthetase of platelets found in Yaroslavl breed calves provides for the generation of a physiologically necessary amount of thromboxane A_2. This was indicated by the results of transfer tests, which showed in the blood plates of the examined calves a slight increase in the activity of both enzymes converting arachidonic acid to thromboxane cyclooxygenase and thromboxane synthetase. Another important mechanism to ensure the propensity to accelerate AP in newborn calves of the Yaroslavl breed should also be considered as a tendency revealed in them to enhance actin formation and myosin formation in response to the appearance of an aggregation inducer in plasma and a tendency to increase secretion from platelets ATP and ADP [30].

In the blood of the examined animals, a tendency to an increase in the number of active forms of platelets was found. This indicated an increase in their sensitivity of platelets to aggregation inducers. In addition, the propensity to increase intravascular platelet activity also indicated a tendency for an increase in the availability of vascular wall collagen for platelets due to a tendency for an increase in the number of free-circulating platelet aggregates in their blood. It also indirectly indicated a slight increase in the blood of newborn calves of the Yaroslavl breed of other aggregation inducers (ADP, thrombin, adrenaline) [28]. The found tendency to growth in the observed calves of the initially low aggregating ability of platelets causes a tendency to increase the content of active forms of platelets and their aggregates of different sizes. This can be considered an important mechanism to minimize the risk of bleeding. The revealed intravascular activity of platelets in newborn calves of the Yaroslavl breed proves the high activity of adhesive and aggregation properties of platelets in the blood and suggests that taking into account the literature [31], apparently due to the presence of a large number of receptors for vascular disaggregant on the membrane.

CONCLUSION

Newborn calves of the Yaroslavl breed are characterized by functional optimality of platelet activity. It provides the physiological conditions required for eliminating the risk of bleeding, as well as optimum microcirculation and an adequate level of metabolism in their tissues. It is possible for them due to the low activity of the mechanisms that ensure platelet adhesion, aggregation and secretion. Non-expressed intravascular platelet activity in newborn calves of the Yaroslavl breed helps to ensure the maintenance of homeostasis in conditions of sufficient blood flow in the capillaries of all internal organs, which creates conditions for their rapid growth and development.

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