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Functional activity of platelets in new-born calves of black-marked breed

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Abstract

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Hemostatic features of platelets in cattle can change depending on many factors of outdoor and internal environment. Estimation of their activity at the beginning of ontogenesis with the account of breed belonging is of great interest. The aim of the research was to determine platelets' activity in new-born calves of black-marked breed. The research involved 32 calves of black-marked breed received from healthy cows after 2nd-3rd in-calf state. The calves were examined on the 1st-2nd, 3rd-4th, 5th-6th, 7th-8th and 9th-10th days of their lives. In our research we applied biochemical, hematological and statistical methods of investigation. In the course of the new-born phase the calves were noted to have a trend to strengthening of platelets' aggregation to all the applied inductors. The quantity of discoid platelets in blood of calves of black-marked breed had a downward trend in the course of observation. The common quantity of activated platelets summarily increased in them by 7.1%. The quantity of circulating in blood little and also average and large platelets' aggregates also had an upward trend in the course of new-born phase. These alterations were provided in calves of black-marked breed by an upward trend of thromboxane synthesis in platelets on behalf of strengthening of cyclo-oxygenase and thromboxane synthetase in them. Besides, it was influenced by content rise of adenosine-phosphates in platelets and a trend to strengthening of their secretion. The content of actin and myosin in inactive platelets of the examined calves increased in the course of new-born phase by 5.2% and 11.4%, respectively. At the same time, they were noted to have a trend to strengthening of actin and myosin additional formation in the course of the process of platelets' aggregation by 6.6% and 8.7%, respectively. Received data allow considering that new-born calves of black-marked breed are characterized by low activity of platelets' aggregation and secretion. It provides physiologically favorable conditions for microcirculation processes in them. Low intravascular platelet activity in these calves promotes the formation of necessary conditions for metabolism in their tissues and, consequently, for their fast growth and development.

Keywords: calves; newborn; black-and-white breed; platelets; aggregation; secretion

Introduction

The degree of functional readiness of hemostasis system mostly determines hemocirculation (Kotova et al., 2017; Zavalishina, 2017b). Platelets have a great significance in this aspect. Their hemostatic activity mostly determines the success of microcirculation (Glagoleva & Zavalishina, 2017b; Skoryatina & Zavalishina, 2017b) in any living beings (Glagoleva & Zavalishina, 2017a; Glagoleva & Zavalishina, 2017c). In previous researches it was noted that platelets' activity could change in the course of active growth (Bikbulatova, 2018b), at aging (Skoryatina & Zavalishina, 2017c; Zavalishina, 2017c), against the background of dysfunctions' development (Shitikova, 2008; Korepano-

va et al., 2015), formation of frank pathology (Skoryatina & Zavalishina, 2017a), appearance of angiopathy (Zaitsev et al., 2017; Zavalishina, 2017a) and in conditions of various medicinal impacts on a body (Lazareva et al., 2005; Bikbulatova, 2017; Bikbulatova & Andreeva, 2017). At the same time, many aspects of platelet hemostasis functioning in cattle are still studied rather poorly. We managed to find only separate works devoted to estimation of platelets' state in these productive animals at some stages of their ontogenesis (Bikbulatova, 2018a). This isolated information doesn't allow drawing a perceptual notion about this issue and forms continuous need of planned researches. The importance of these researches is provided by high signification of platelets' activity for capillary bloodstream. It is acknowledged that microcirculation determines the intensity of growth and development of animals and their separate organs realizing their productive potential (White & Rompietti, 2007). Taking into account the presence of genetic differences between breeds of cattle and great physiological signification of platelets' activity for realization of productive potential, it was very important to conduct estimation of platelets' functional peculiarities in calves of highly productive (according to the level of milk productivity) black-marked breed at the start of their functional peculiarities' realization in ontogenesis.

Aim: to determine platelets' activity in new-born calves of black-marked 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 on March 18, 1986, and confirmed in Strasbourg on June 15, 2006) and approved by the local ethic committee of Russian State Social University (Record №11, dated December 4, 2015).

The research was conducted at the calf-house of the farm "Kolos" situated in Kursk region (Russia) in 2016. It involved 32 calves of black-marked breed received from healthy cows after 2^{nd}-3^{rd} in-calf state. The calves were observed and examined 5 times in the course of new-born phase: on the 1^{st}-2^{nd}, 3^{rd}-4^{th}, 5^{th}-6^{th}, 7^{th}-8^{th} and on the 9^{th}-10^{th} days of life.

Indirect estimation of intensity of thromboxane synthesis in platelets and enzymatic activity of participating in this process cyclo-oxygenase and thromboxane synthetase was conducted in three transfer tests with the help of photoelectrocolorimeter (Levi, 2005). We determined the quantity of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) in platelets, activity of their secretion at the appearance of collagen in the medium and the levels of actin and myosin in protein cytoskeleton of intact and activated platelets under the impact of ADP (Levi, 2005).

The time of platelets' aggregation (PA) was estimated with the help of visual micromethod (Skorjatina, 2018) using as inductors ADP (0.5×10^{-4} M), collagen (dilution 1:2 of the basic suspension), adrenaline (5.0×10^{-6} M), thrombin (0.125 un/ml) and ristomicin (0.8 mg/ml) in standardized according to the number of platelets plasma till 200×10^{9} pl. The level of intravascular platelets' activity (IPA) was determined in the course of phase-contrast microscopy (Oshurkova & Glagoleva, 2017). Statistical processing of received information was made with the help of a program packet "Statistics for Windows v. 6.0", "Microsoft Excel". Differences in data were considered reliable in case of $p<0.05$.

Results of Research

New-born calves of black-marked breed were found to have a trend to strengthening of initially low platelets' functional activity. So, PA developed in taken under observation calves on the 1^{st}-2^{nd} days of life in response to collagen during 32.2±0.12 s, later accelerating by the 9^{th}-10^{th} days of life till 31.5±0.10 s (Table 1). Similar trend to acceleration of PA process was detected in response to ADP and ristomicin till 39.7±0.11 s and 47.4±0.18 s, respectively. At the same time, PA with thrombin and adrenaline also had a trend to time reduction of its coming till 52.6±0.14 s and 97.0±0.18 s.

The quantity of platelets-discocytes in blood of the examined calves had a downward trend in the course of the new-born phase. During the period of calves' observation the sum of platelets' active forms in their blood had an upward trend by 7.1%. The number of circulating in their blood platelets' aggregates of different sizes also had an upward trend during the first 10 days of life.

Found trend to the growth of thromboxane synthesis in calves' platelets can be considered an important mechanism forming a trend to PA strengthening in new-born calves of black-marked breed. It was indirectly judged by PA rise in a simple transfer test which was equal to 30.5±0.08% on the 9^{th}-10^{th} days of life. An upward trend of cyclo-oxygenase and thromboxane synthetase activity in calves' platelets lay in the basis of it in the observed calves. It was pointed by degree rise of PA reduction in collagen-aspirin test indirectly estimating cyclo-oxygenase activity in platelets (by the end of observation – 79.8±0.10%). The level of PA reduction in collagen-imidazole test indirectly estimating thromboxane synthetase activity in calves' platelets, also rose and was equal to 41.1±0.07% on the 9^{th}-10^{th} days of life.

Initially low ATP and ADP content in calves' platelets had an upward trend reaching by the 9^{th}-10^{th} days of life

5.50±0.015 and 3.30±0.006 μmol /10^9 platelets. In these conditions the activity of their secretion out of platelets in the course of the first 10 days of life had a trend to weakening till 30.6±0.20% and 40.7±0.14%.

Actin and myosin content in inactive platelets of calves on the 1st-2nd days of their life was equal to 26.5±0.14% and 12.3±0.17% of common protein in a platelet, and by the end of observation – 27.9±0.16% and 13.7±0.19% of common protein in a platelet. In the process of calves' observation we also found an upward trend of actin and myosin formation in conditions of platelets' aggregation.

Discussion

Modern researches allowed acknowledging high biological signification of hematological researches on animals

Table 1. Thrombocyte indices in newborn calves of black and motley breeds

Indicators	Calves of black and motley breed, n=32, M±m				
	1-2 day	3-4 day	5-6 day	7-8 day	9-10 day
Content of ATP in platelets before the onset of secretion, μmol /10^9 platelets	5.44±0.012	5.45±0.014 P < 95%	5.47±0.010 P < 95%	5.49±0.010 P < 95%	5.50±0.015 P < 95%
Content of ADP in platelets before the onset of secretion, μmol / 10^9 platelets	3.20±0.008	3.22±0.007 P < 95%	3.24±0.009 P < 95%	3.27±0.010 P < 95%	3.30±0.006 P < 95%
Level of ATP secretion, %	28.6±0.12	28.9±0.16 P < 95%	29.4±0.19 P < 95%	30.0±0.15 P < 95%	30.6±0.20 P < 95%
Level of ADP secretion, %	38.0±0.14	38.4±0.17 P < 95%	38.9±0.20 P < 95%	39.5±0.18 P < 95%	40.7±0.14 P < 95%
Degree of recovery of platelet aggregation during collagen-aspirin test,%	78.3±0.09	78.7±0.12 P < 95%	78.8±0.09 P < 95%	79.3±0.09 P < 95%	79.8±0.10 P < 95%
Degree of restoration of platelet aggregation in the collagen-imidazole assay,%	39.0±0.07	38.9±0.09 P < 95%	39.6±0.06 P < 95%	40.2±0.08 P < 95%	41.1±0.07 P < 95%
Platelet aggregation activity in a simple transfer sample,%	29.7±0.05	29.9±0.06 P < 95%	30.2±0.04 P < 95%	30.3±0.06 P < 95%	30.5±0.08 P < 95%
Content of actin in inactive platelets,% of the total protein in platelets	26.5±0.14	26.7±0.12 P < 95%	26.8±0.08 P < 95%	27.6±0.11 P < 95%	27.9±0.16 P < 95%
Content of actin in platelets with ADP-aggregation,% of the total protein in platelets	36.3±0.10	36.9±0.16 P < 95%	37.4±0.10 P < 95%	38.1±0.17 P < 95%	38.7±0.14 P < 95%
Content of myosin in inactive platelets,% of the total protein in thrombocytes	12.3±0.17	12.7±0.12 P < 95%	13.2±0.15 P < 95%	13.5±0.19 P < 95%	13.7±0.19 P < 95%
Myosin content in platelets with ADP aggregation,% of total protein in platelets	26.3±0.10	26.9±0.16 P < 95%	27.4±0.12 P < 95%	28.0±0.14 P < 95%	28.6±0.18 P < 95%
Time of onset of platelet aggregation with ADP, s	41.2±0.15	40.9±0.10 P < 95%	40.4±0.12 P < 95%	40.2±0.08 P < 95%	39.7±0.11 P < 95%
Time of onset of platelet aggregation with collagen, s	32.2±0.12	32.0±0.15 P < 95%	31.8±0.14 P < 95%	31.6±0.10 P < 95%	31.5±0.10 P < 95%
Time of onset of platelet aggregation with thrombin, s	53.6±0.17	54.0±0.19 P < 95%	53.5±0.16 P < 95%	53.2±0.12 P < 95%	52.6±0.14 P < 95%
Time of onset of platelet aggregation with ristomycin, s	49.6±0.18	48.3±0.22 P < 95%	48.0±0.19 P < 95%	47.7±0.15 P < 95%	47.4±0.18 P < 95%
Time of the onset of platelet aggregation with epinephrine, s	99.6±0.24	98.5±0.20 P < 95%	97.9±0.25 P < 95%	97.3±0.17 P < 95%	97.0±0.18 P < 95%
Number of platelets-discocytes, %	78.8±0.16	78.5±0.14 P < 95%	77.9±0.12 P < 95%	77.6±0.13 P < 95%	77.3±0.18 P < 95%
Sum of active forms of platelets, %	21.2±0.15	21.5±0.13 P < 95%	22.1±0.15 P < 95%	22.4±0.19 P < 95%	22.7±0.16 P < 95%
Number of small platelet aggregates, per 100 free platelets	3.2±0.09	3.2±0.06 P < 95%	3.3±0.05 P < 95%	3.2±0.07 P < 95%	3.5±0.04 P < 95%
Number of medium and large platelet aggregates per 100 free platelets	0,10±0,026	0.11±0.025 P < 95%	0.11±0.019 P < 95%	0.11±0.025 P < 95%	0.11±0.023 P < 95%

Note: the reliability of the dynamics of indicators in relation to 1-2 days age was not found.

and human beings as they can help to find out many aspects of functioning of homeostasis maintenance mechanisms in mammals (Shmeleva et al., 2018). Notwithstanding the great significance for functioning of youngsters' bodies of high-productive cattle breeds platelet activity is still studied rather poorly. It served the reason for conducting the present research on black-marked breed.

In the result of estimation of calves' PA in response to collagen and ristomicin we detected their initially low platelets' ability to adhesion in the course of the new-born phase but it had an upward trend. Evidently, two mechanisms lay in the basis of it (Morozova et al., 2018). We could speak about the first mechanism basing on the found trend to acceleration of blood platelets' aggregation in response to collagen. It could be connected with gradual rise of the quantity of receptors to collagen – glycoproteins Ia – IIa and VI – on the surface of calves' platelets in the course of the new-born phase. As the second mechanism of strengthening of platelets' adhesion in calves of black-marked breed we could consider the increase of receptors to von Willebrand's Factor (GPIb) on their surface at simultaneous increase of this substance in their blood. It was pointed by detected in the examined calves PA acceleration with ristomicin.

Found in new-born calves of black-marked breed trend to acceleration of platelets' aggregation, evidently, provides high protection of their bodies from loss of blood. Little acceleration of their PA in response to strong inductors of aggregation (collagen and thrombin) is, evidently, connected with some increase of receptors to them on platelets at little activation of phospholipase C and phosphoinositol way and light stimulation of phospholiration of contractile system proteins. An upward trend of inositoltriphosphate formation in platelets of calves of black-marked breed was provided, evidently, by little strengthening of Ca^{2+} yield out of depo being the cause of their stimulation rise of the process of actomyosin self-assembly and reduction (Skoryatina et al., 2017).

Weak inductors of platelets' aggregation (ADP and adrenaline) in calves of black-marked breed also caused platelets' aggregation having a trend to acceleration. It was, evidently, provided by the rise of receptors' density to them on platelets' surface, physiological strengthening of expression of fibrinogenic receptors (GPIIb-IIIa) and some activity rise of phospholipase A_2 in them. The last mechanism provided the yield of arachidonic acid definite quantity out of membrane phospholipids what promoted a trend to strengthening of thromboxane A_2 synthesis (Morozova et al., 2018). Besides, found in calves of black-marked breed upward trend of functional capacities of platelets' cyclo-oxygenase and thromboxane synthetase provided generation of thromboxane A_2 physiologically necessary quantity. It was pointed by the results of transfer tests which showed little activity strengthening of both enzymes of arachidonic acid conversion into thromboxane – cyclo-oxygenase and thromboxane synthetase – in blood platelets of the examined calves. There is one more significant mechanism of provision of a trend to PA acceleration in new-born calves of black-marked breed. It is found in them trend to strengthening of actin-formation and myosin-formation in response to the appearance of aggregation inductor in plasma and trend to secretion increase of ATP and ADP out of platelets.

To study peculiarities of initial stages of platelets' activation in new-born calves of black-marked breed *in vivo* we applied in our research the method of IPA estimation with the help of phase-contrast microscopy. In blood of the examined animals we found a trend to quantity rise of platelets' active forms. It pointed at the rise of their platelets' sensitivity to aggregation inductors. Besides, the trend to IPA rise also pointed at the presence of an upward trend of availability of vascular wall collagen for platelets because of a trend to quantity increase of freely circulating platelets' aggregates in their blood. It also indirectly pointed at little increase of other aggregation inductors (ADP, thrombin, adrenaline) in blood of new-born calves of black-marked breed (Skoryatina et al., 2017). Found in the observed calves upward trend of initially low platelets' aggregating capacity causes a trend to content increase of platelets' active forms and their aggregates of different sizes. It can be considered an important mechanism of bleeding risk minimization at minimum blockade risk of functionally significant microvessels' number by platelets' aggregates and preservation of platelet-vascular interaction mechanisms on the level of physiological optimum. The detected intravascular platelets' activity in new-born calves of black-marked breed proves high perfection of platelets' adhesive and aggregative capacities in blood and gives the basis to suppose (taking into account literature information (Zaitsev et al., 2017; Shmeleva et al., 2018) the presence of physiologically sufficient capacity to disaggregation in them, evidently, on behalf of the presence of a great number of receptors to vascular disaggregants on membranes.

Conclusion

New-born calves of black-marked breed are characterized by functional sufficiency of platelet hemostasis activity. It provides physiologically necessary conditions for bleeding prevention in them and optimal conditions for microcirculation and metabolism in their tissues. It is realized in them by low activity of mechanisms of platelet adhesion, aggregation and secretion. Little intravascular platelets' activity in new-

born calves of black-marked breed mostly provides homeostasis maintenance at provision of bloodstream efficiency in capillaries and optimum of metabolism in their muscles and internals what promotes fast growth and development of animals of this breed.

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