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Physiological Features Of Platelet Functioning In Calves Of Holstein Breed During The Newborn.

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ABSTRACT

The level of platelet activity in cattle strongly determines the state of animal metabolic processes in animals, which is especially important at the beginning of ontogenesis and can vary in different breeds. The study was conducted on 42 Holstein calves derived from healthy cows with normal pregnancy. Calves were examined and examined for 1-2, 3-4, 5-6, 7-8 and 9-10 days of life. Used biochemical, hematological and statistical research methods. During the neonatal phase, the calves showed a tendency to weaken platelet aggregation in response to all inductors used. The concentration of discocytes in the blood of Holstein calves during the neonatal phase experienced a tendency to increase. The sum of the active forms of platelets they had a tendency to decrease by 8.2%. The number of small and large platelet aggregates freely moving through the blood also tended to decrease during the first 10 days of ontogenesis. Apparently, this was provided in calves with a tendency to weaken the synthesis of thromboxane in platelets and a decrease in the content of adenosine phosphates in them with a tendency to weaken their secretion. The amount of actin and myosin on the 1-2 day in the platelets of the observed calves was small and tended to decrease during the neonatal phase. Additional education of actin and myosin on the background of platelet aggregation in them also experienced a tendency to decrease during the observation. It can be considered that high functional perfection of platelet hemostasis is peculiar to newborn Holstein calves, which provides very physiologically favorable conditions for microcirculation. This is ensured by their low activity of the mechanisms that realize platelet adhesion, aggregation and secretion. A small intravascular activity of platelets in newborn calves of Holstein breed provides optimum perfusion and metabolism in all internal organs necessary for the rapid growth and development of animals.

Keywords: calves, newborn phase, holstein breed, platelets, aggregation, secretion.

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INTRODUCTION

The level of functionality of the hemostasis system largely determines the success of hemocirculation [1,2]. In this aspect, platelets are very important, the level of activity of which largely determines the state of microcirculation [3,4] in various biological objects [5, 6]. It was noted that the functional characteristics of platelets may change during growth and development [7], during aging [8,9], with the development of various dysfunctions [10,11], the appearance of somatic pathology in the body [12], the occurrence of vascular disorders [13,14] and during various therapeutic measures [15,16,17]. However, various aspects of platelet hemostasis remain poorly studied in cattle. There are only separate works on platelet activity in these productive animals at some stages of their ontogenesis [18]. This does not allow for a holistic picture of it and dictates a great need for further research. However, there is reason to believe that it is precisely the level of activity of platelets, including cattle, that largely determines the intensity of capillary blood flow, the rate of development of the structures of the organism and the level of their functional activity based on the implementation of hereditary information in the course of ontogeny [19,20]. In view of the presence of genetic and physiological differences between cattle breeds, it was of great interest to evaluate platelet activity in calves of a very productive in terms of the milkiness level of the Holstein breed during the neonatal phase.

The goal is to assess the state of platelet activity in healthy Holstein calves during the neonatal phase.

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).

The work was performed on 42 Holstein calves obtained from healthy cows with normal pregnancy. 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.

An indirect assessment of the level of thromboxane synthesis in platelets and the indirect determination of cyclooxygenase and thromboxane synthetase activity in them was carried out using three transfer tests, in which platelet aggregation was determined on a photoelectrocolorimeter. The quantitative content of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) in platelets, the level of their secretion in response to collagen and the level of actin and myosin in the cytoskeleton of inactive platelets and in platelets during their aggregation under the influence of ADP were determined.

Platelet aggregation (AP) was determined using a visual micromethod using as inducers ADP ($0.5x10^{-4}$ M), collagen (dilution 1:2 of the main suspension), thrombin (0.125 units / ml), adrenaline (5.0×10^{-6} M) and ristomycin (0.8 mg/ml) in plasma with a standardized number of platelets up to 200x109 platelets. Intravascular platelet activity (IPA) was determined using phase-contrast microscopy.

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

The Holstein calves surveyed in the work during the neonatal phase showed a tendency to decrease of the initially low platelet activity. Thus, in the examined animals, at 1-2 days of life, AP came in response to collagen in 37.5±0.15s, subsequently slowing down by 9-10 days of life to 38.3±0.10s. A similar tendency to slow AP was found in response to ADP and ristomycin to 47.5±0.14 s and 55.7±0.20s, respectively. There was also a tendency to inhibit AP with thrombin (up to 59.7±0.21 s) and adrenaline (up to 107.9±0.24 s).

The number of discocytes in the blood of the observed calves experienced a tendency to increase during the neonatal phase. At the same time, during the time of the observation, the sum of the active forms of platelets they had a tendency to decrease by 8.2%. The number of small and large platelet aggregates that freely move through the blood also tended to decrease in their first 10 days of ontogenesis.

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Table. Platelet activity in newborn calves of Holstein breed

Indicators under consideration	Calves Holstein breed, n=42, M±m				
	1-2 day	3-4 day	5-6 day	7-8 day	9-10 day
value of platelets' aggregation reduction in collagen-aspirin test, %	75.8±0.12	75.4±0.16	74.6±0.08	73.8±0.10	73.2±0.07
the level of platelets' aggregation reduction in collagen-imidazole test, %	36.4±0.05	36.1±0.04	35.7±0.07	35.2±0.09	34.6±0.11
platelets' aggregation lowering in a simple transfer test, %	26.5±0.06	26.0±0.08	25.7±0.09	25.3±0.08	25.2±0.10
Content of ATP in platelets prior to secretion, umol/10^9 platelets	5.33±0.012	5.29±0.010	5.27±0.014	5.27±0.010	5.23±0.008
The maintenance of ADP in platelets prior to secretion, umol/10^9 platelets	3.18±0.006	3.17±0.007	3.16±0.005	3.15±0.008	3.13±0.009
Secretion level ATP,%	25.4±0.12	25.3±0.14	25.1 ±0.12	24.8±0.10	24.5±0.14
Secretion level ADP,%	32.6±0.10	32.5±0.16	32.4±0.09	32.1±0.15	31.7±0.12
The number of actin in inactive platelets,% of total protein in platelets	20.7±0.10	20.5±0.12	20.3±0.08	20.1±0.05	20.0±0.07
The number of actin in platelets with ADP-aggregation,% of total protein in platelets	32.2±0.10	32.1±0.14	32.0±0.09	31.8±0.07	31.7±0.12
Number of myosin in inactive platelets,% of total protein in platelets	9.9±0.14	9.7±0.15	9.6±0.08	9.4±0.09	9.1±0.12
Number of myosin in platelets with ADP-aggregation,% of total protein in platelets	22.1±0.14	22.0±0.10	21.8±0.08	21.7±0.07	21.6±0.09
Aggregation of platelets with ADP,s	46.6±0.13	46.7±0.15	46.9±0.16	47.3±0.12	47.5±0.14
Aggregation of platelets with collagen, s	37.5±0.15	37.8±0.12	37.9±0.16	38.2±0.09	38.3±0.10
Aggregation of platelets with thrombin, s	58.7±0.17	58.9±0.20	59.1±0.23	59.5±0.19	59.7±0.21
Aggregation of platelets with rhystomicin, s	54.8±0.16	55.0±0.15	55.2±0.25	55.4±0.28	55.7±0.20
Aggregation of platelets with adrenalin, s	106.8±0.24	107.0±0.27	107.2±0.19	107.4±0.26	107.9±0.24
Thrombocytes-discocytes, %	84.2±0.16	84.4±0.24	84.8±0.20	85.1±0.18	85.4±0.23
Sum of thrombocytes active forms, %	15.8±0.15	15.6±0.13	15.2±0.19	14.9±0.14	14.6±0.20

Number of little aggregates (in 100 free thrombocytes)	2.6±0.07	2.6±0.03	2.5±0.05	2.5±0.06	2.4±0.08
Number of medium and large aggregates (in 100 free thrombocytes)	0.09±0,024	0.08±0.019	0.07±0.015	0.07±0.012	0.06±0.017

Note: the reliability of the dynamics of indicators in relation to 1-2 daily age is not detected.

Apparently, the development trend of AT weakening in Holstein calves during the neonatal phase is based on the revealed tendency to weaken their synthesis in thromboxane platelets, which was indirectly judged by a decrease in AT in a simple transfer test (at 9-10 days of life 25.2 ± 0.10%). This was ensured in the observed animals with a tendency to a decrease in the activity in their platelets of both enzymes of its synthesis, cyclooxygenase and thromboxane synthetase. The level of AT recovery in the collagen-aspirin test, which indirectly characterizes the activity of cyclooxygenase in platelets, reached 73.2 ± 0.07% by the end of the observation. At the same time, the level of AT recovery in the collagen-imidazole sample, which makes it possible to indirectly evaluate the activity of thromboxane synthetase in platelets, in the observed calves also decreased during observation, amounting to 9-10 days to 34.6 ± 0.11%.

At the beginning of the observation, the content in the platelets of calves of ATP and ADP, which was low, tended to decrease, reaching 5.23±0.008 and 3.13±0.009 µmol /10^9 platelets by the end of the neonatal phase. The levels of their secretion from platelets during the observation period experienced a tendency to weaken by 3.4% and 2.8%, amounting to 24.5±0.14 and 31.7±0.12% by the end of the observation.

The content of actin and myosin in inactive platelets for 1-2 days was 20.7±0.10 and 9.9±0.14% of the total protein in the platelet, and by the end of the neonatal phase 20.0±0.07 and 9.1±0.12% of the total protein in the platelet. Additional education of actin and myosin against the background of platelet aggregation in calves of Holstein breed also experienced a tendency to decrease during the entire neonatal phase.

DISCUSSION

Hematological studies are now being conducted very actively. Their results may help further uncover many aspects of the regulatory mechanisms in mammals [21,22]. Despite the great physiological significance of platelet activity in young high-yielding breeds of cattle, it remained very poorly studied. In this work, the characteristics of platelet activity in newborn calves belonging to the Holstein breed were first identified.

According to the results of assessment in calves AT with collagen and ristomycin, it was possible to ascertain the initially low adhesive ability of their platelets, which have a tendency to decrease during the neonatal period. Apparently, this was ensured by at least two mechanisms [23,24]. The first mechanism was identified by a tendency to inhibit the aggregation of platelets in response to collagen [25]. It is associated with the development of a tendency to decrease the initially low number of collagen receptors - glycoproteins Ia - IIa and VI on the membranes of calf platelets [26] during the first 10 days of their life. The presence of a second mechanism for ensuring low platelet adhesion potential was detected in Holstein calves in terms of a decrease in AT with ristomycin [27]. This mechanism was associated with a tendency to decrease in the initial concentration of von Willebrand factor in their blood during the neonatal phase and due to this weak involvement in the adhesion process of the receptors to it (GPI in) on the platelet membranes [28,29].

It was found that the development of a tendency to slow down the initially low platelet aggregation, which is capable of very positively influencing the microcirculation process in tissues, is characteristic of new Holstein calves for newborn calves [30]. Under conditions of initially low sensitivity of platelets to aggregation inductors, which has a tendency to decrease, the combination of strong aggregation inductors, collagen and thrombin with their own receptors, was inhibited [31,32]. This contributed to the inhibition of the activity of phospholipase C and the phosphoinositol pathway with an unexpressed phosphorylation of proteins of the contractile system [33]. The inactive formation of inositol triphosphate in their platelets provided, according

to the literature, restraining the release of Ca2 + from its depot and, to some extent, causing a weakening of the process of self-assembly and reduction of actomyosin in them [34,35].

Functionally weak inducers of platelet aggregation (ADP and adrenaline) also caused low aggregation in Holstein calves, which had a tendency to weaken during observation [35]. This was apparently provided by a small number of receptors for them on platelet membranes, physiologically minimal expression of fibrinogen receptors (GPIIb-IIIa) and a low stimulation of phospholipase A_2 activity during the aggregation process [36]. The latter mechanism allowed a limited amount of arachidonic acid to escape from membrane phospholipids and contributed to the inhibition of thromboxane A_2 synthesis [37]. In addition, low functionality of cyclooxygenase and thromboxane synthetase of platelets were observed in Holstein calves, which also ensured the generation of a physiologically minimal amount of thromboxane A_2 [38]. This was proved by the results of the transfer tests that revealed in the blood plates of Holstein calves a low activity of both enzymes converting arachidonic acid to thromboxane cyclooxygenase and thromboxane synthetase [39]. An important mechanism for ensuring low AT in newborn Holstein calves should also be considered as a low actino and myosin formation found in response to the effects of aggregation inducer and moderate platelet secretion of ATP and ADP [40].

A small amount of active forms of platelets in the blood of animals examined during the study confirmed their reduced sensitivity to aggregation inducers [41]. The low level of IPA also indicated the presence of low availability of collagen of the vascular wall due to the high endothelium safety as a result of a small amount of freely circulating active platelet forms and their aggregates in the blood of animals. It also indirectly indicated a weak contact of these fibers with blood plates and a low level in the blood of Holstein calves of other aggregation inducers (ADP, thrombin, adrenaline) [42]. The observed tendency to decrease in the observed animals of the low aggregating ability of platelets causes a tendency to a decrease in the content of their active forms and their dynamic aggregates of different sizes [43]. This circumstance should be considered as an important mechanism for restraining the activity of platelet hemostasis, minimizing the risk of blockade of a functionally significant number of capillaries by platelet aggregates and maintaining the optimal level of platelet-vascular relationships [44]. The low intravascular activity of platelets in newborn Holstein calves indicates not only the low activity of the adhesive and aggregation capabilities of platelets in vivo, but also suggests, based on literature data [45], that they have a high physiologically very beneficial disaggregating ability, apparently related with hypersensitivity of their receptors to vascular antiagregant.

CONCLUSION

The newborn Holstein calves have a large functional perfection of platelet hemostasis, which significantly provides physiologically favorable conditions for the flow of microcirculation. This is ensured by their low activity of the mechanisms that realize platelet adhesion, aggregation and secretion. Non-expressed intravascular platelet activity in newborn calves of Holstein breed creates the conditions for optimum perfusion and metabolism in all internal organs necessary for their rapid growth and development.

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