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Functional Platelet Activity In Heifers In Growing.

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ABSTRACT

The process of ontogenesis of animals is inevitably accompanied by a regular morphofunctional dynamics of all systems of their body, including blood. Currently, an understanding of the great importance of the hemostasis system in the normal formation of the adaptive capabilities of the organism has been achieved. In heifers during rearing, there is a tendency to weakening the intensity of lipid peroxidation to increase the activity of antioxidant protection of platelets, enhance the actin-myosin mechanism with a high quantitative content of adenosine phosphates in them and pronounced their secretion during activation and aggregation. This combined in the observed heifers with a tendency to increase the functional activity of platelets, recorded in vitro and in vivo. It is likely that a slight increase in platelet activity in heifers during rearing is associated with the maturation of the receptor and post-receptor mechanisms of platelets, which determine the functional readiness of platelets in response to the inevitable increase in environmental effects on the animal's body.

Keywords: heifers, rearing, platelets, aggregation, secretion.

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INTRODUCTION

Ontogenesis of mammals is always accompanied by serious changes in the morphological and functional parameters of their organism [1-4]. This is true for all their body systems, including blood [5,6]. At present, an understanding of the great importance of the hemostasis system in the normal formation of the adaptive capabilities of the organism has been achieved [7, 8]. At the same time, an important role in creating optimal conditions for microcirculation [9,10], necessary for growth, development and the maximum possible manifestation in the phenotype of the productive properties of animals, is played by the blood plates due to their peculiarity to aggregation, which affects the fluidity of blood and, most, on its influx to the tissues [11-15].

Insemination, as a rule, in heifers is preceded by a period of rearing, during which the final maturation of all their organs and systems occurs and the growth of the organism is completed [16]. At the same time, in spite of the significance of the time interval in the development of the animal, the aggregation capacity of the platelets remains very insufficiently studied in them. Taking this into account, the purpose of this study was to find out the peculiarities of platelet activity in healthy heifers during growing.

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).

Under observation were taken 42 heifers that are growing, the state of which was taken into account at the age of about 12 months, about 13 months, about 14 months. and about 15 months of life. In all animals, the platelets were washed and resuspended, followed by an assessment of the levels of malondialdehyde (MDA) and acylhydroperoxides (AHP). The functional capabilities of intra-platelet anti-oxidation enzymes - catalase and superoxide dismutase were evaluated. The platelets revealed the content of adenosine triphosphate and adenosine diphosphate with an estimate of the magnitude of their secretion under the influence of collagen. The protein composition of the cytoskeleton of blood platelets (actin and myosin) was established under conditions of platelet activation and aggregation with ADP and thrombin. Platelet aggregation (AP) was recorded by a visual micromethod with: ADP (0.5 ± 10^{-4} M), collagen (1: 2 dilution of the main suspension), thrombin (0.125 U/ml), ristomycin (0.8 mg/ml), H_2O_2 (7.3×10^{-3} M), adrenaline (5×10^{-6} M) and their combinations (ADP and adrenaline; ADP and collagen; adrenaline and collagen; ADP and thrombin; ADP, collagen and adrenaline; ADP, thrombin and adrenaline; ADP, collagen, thrombin and adrenaline). The intravascular activity of platelets was established with a phase contrast method. Statistical calculation of the results was carried out by Student's t-criterion.

RESULTS OF THE STUDY AND DISCUSSION

All the chicks during the study were healthy.

The content of AHP in the blood plates of heifers at the age of about 12 months amounted to 2.66 ± 0.15 D₂₃₃/10⁹ platelets, experiencing during the observation a slight downward trend, reaching 15 months life 2.58 ± 0.16 D₂₃₃/10⁹ platelets. The amount of MDA in their platelets at 12 months life was 0.75 ± 0.10 nmol/10⁹ platelets, also experiencing a slight tendency to decrease during follow-up (in 15 months of life 0.69 ± 0.008 nmol/10⁹ platelets).

The activity of catalase and platelet superoxide dismutase in the observed animals gradually increased during the observation period from 10120.2 ± 9.90 IU/10⁹ platelets to 10380.0 ± 8.06 IU/10⁹ platelets and from 1910.2 ± 3.12 IU/10⁹ platelets to 2052.4 ± 2.70 IU/10⁹ platelets, respectively.

During the observation period, the content of ATP and ADP in platelets of healthy heifers experienced a slight tendency to increase from 5.80 ± 0.20 μ mol/10⁹ platelets to 5.85 ± 0.18 μ mol/10⁹ platelets and from

3.63±0.09 $\mu\text{mol}/10^9$ platelets to 3.69±0.12 $\mu\text{mol}/10^9$ platelets, respectively). The expression of ATP and ADP secretion under the action of collagen from their platelets remained unchanged.

The amount of actin in intact platelets in healthy 12 months heifers corresponded to 37.1±0.17% of the total protein in the platelet, amounting to 15 months life of 37.9±0.10% of the total protein in the platelet. The severity of additional formation of actin in heifers with activation of the blood platelets by a strong or weak inducer and with their aggregation also experienced a slight tendency to increase.

Similar dynamics of activity in platelets of the observed heifers was also detected for the myosin mechanism. It is noted that in non-activated blood platelets of heifers about 12 months life the amount of myosin reaches 17.3±0.10% of the total protein content in the platelet, experiencing a tendency to increase in the future and amounting to 15 months life of 18.4±0.12% of the total protein content in the platelet. Against the background of platelet activation and aggregation by strong or weak inducers in healthy heifers, during the entire rearing period, there was a slight tendency to increase the severity of additional self-assembly of myosin.

In observable heifers, starting from 12 months age, there is a slight tendency to reduce the time of development of antibodies with all the inductors used and their combinations.

AP in response to collagen developed at the beginning of the observation for 23.7±0.18 s, slightly accelerating towards its end. The tendency to reduce the time of AP development in the observed animals was also observed under the influence of ADP and ristomycin. A little more slowly, AP occurred with H_2O_2 , thrombin and adrenaline, the development time of which also tended to decrease for rearing. The found tendency to accelerate AP in heifers at rearing when evaluating AP with one inductor was consistent with the established fact of accelerating AP when it was determined in conditions of using two or three agonists simultaneously.

The age dynamics of platelet activity found in an in vitro evaluation was confirmed by the results of the study of the intravascular platelet activity. Thus, the number of discocytes in the blood of heifers at 12 months life was 71.0 ± 0.19%, gradually experiencing a slight downward trend until the end of observation (at 15 months 70.1±0.26%). The total content of active forms of platelets gradually increased during the observation time by only 13.0%. In the blood of the heifers, during the observation period, the number of free-moving small and large platelet aggregates gradually increased from 5.8±0.07 and 0.22±0.08 per 100 free-lying platelets at the beginning of the observation to 6.5±0.19 and 0.28±0.03 per 100 free-lying platelets at its end, respectively. The number of platelets included in the aggregates in heifers during the observation increased by 8.8%.

The process of optimal functioning of the body is possible with an adequate flow of nutrients and oxygen to the tissues, which is largely due to the optimality of the rheological properties of blood cells that inevitably change during ontogenesis [17]. An important role in adequate blood rheology in animals is played by platelet activity, which also experiences ontogenetic dynamics [18,19].

In the present work, it was found that for healthy heifers in growing, the antioxidant protection of platelets gradually increases, effectively controlling lipid peroxidation in them. The low activity of free radical processes during rearing in heifers largely ensures the optimal functioning of the mechanisms of activation of blood platelets found in them from 12 to 15 months life, including the optimality of the process of self-assembly of the actin-myosin complex and the quantitative content of platelets and secretion of them ADP and ATP.

Acceleration during the observation period of platelet aggregation with strong aggregation inducers - collagen and thrombin indicated the activation of phospholipase C in them, providing a phosphoinositol pathway for stimulating platelets by increasing the amount of diacylglycerol and protein kinase C with the intensification of ametamid and akinbey. Reducing the time of platelet aggregation with weak aggregation inducers - ADP and adrenaline indicated an increase in the availability of receptors to them and / or an increase in their number on the surface of platelets with increased expression of fibrinogen receptors (GP1Ib-IIIa) and an increase in the functional capacity of A_2 phospholipase [22-24], providing arachidonic acid release from platelet membranes for the synthesis of thromboxane A_2 . The revealed slight acceleration of antibodies

in response to the simultaneous use of two or three inductors indicated in teens on rearing the age tendency to enhance their synergistic effects on platelets, simulating real conditions in vivo [25]. The found tendency to increase intravascular platelet activity during rearing in heifers also indicated increased expression of fibrinogen receptors (GPIIb-IIIa) on their membranes [26], confirming the increased sensitivity of their surface receptors to aggregation inducers (ADP, thrombin, adrenaline) obligatory in the blood (ADP, thrombin, adrenaline) and activation of intrathrombocyte aggregation mechanisms [27-31].

CONCLUSION

During rearing, heifers tend to increase the ability of platelets to aggregate, the sum of their activated forms and freely moving aggregates of all sizes through blood, resulting from the interaction of environmental influences on the body with its adaptive responses.

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