ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Functional Features Of Platelet Aggregation In Heifers Of The Ayrshire Breed, Which Are Being Prepared For Insemination.

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**ABSTRACT**

Individual development in cattle is always associated with the dynamics of systems that regulate and integrate their body. To which number the blood belongs. Of great importance in the preservation of liquid blood properties is platelet aggregation, which greatly affects the state of microcirculation, anabolism and productivity, especially in highly productive cows. Objective: to evaluate the features of platelet aggregation in heifers of the Ayrshire breed, preparing for insemination. 36 heifers of the Ayrshire breed, cultivated at the age of 12.15 and 18 months, were examined. Hematological and statistical methods of investigation were used in the work. The strongest aggregation of platelets was noted in animals for adenosine diphosphate. Collagen and ristomycin aggregation were less pronounced and had the same directionality to enhancement. The aggregate platelet aggregation index with three tested aggregation inducers at the end of the observation was 25.6 ± 0.05%, 10.0 ± 0.26% and 10.3 ± 0.39%, respectively. This indirectly indicated a high integrity in heifers of the vascular endothelium and a small amount in their blood of the von Willebrand factor. The ability to disaggregate their platelets with all tested inductors in heifers preparing for insemination tended to increase, balancing the increase in aggregation. The low activity of platelets in heifers of the Ayrshire breed before insemination should be considered a necessary condition for the optimum of anabolic processes in their growing organism and the foundation of their future productivity. The results obtained in the study can be used in future studies on the cattle of the Ayrshire breed as normative.

**Keywords:** platelets, aggregation, heifers, rearing, Airshire breed.

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ISSN: 0975-8585

## INTRODUCTION

Individual development of the animal is associated with the dynamics of the activity of systems regulating its organism, including blood [1,2]. Physiologically, its biological subsystem providing, on the one hand, the preservation of its liquid state, and on the other hand, the prevention and relief of bleeding, is hemostasis [3,4]. Being a complex system, hemostasis consists of a number of functionally significant components, very important of which are platelets [5,6], which can influence its other mechanisms [7]. The effectiveness of blood platelets, the prevention of thrombosis, ischemia and infarctions of organs, the control of hemorrhages, the degree of protection against dissemination of bacteria and toxins from the lesions around the body depend to a large extent on the functional activity of platelets [8,9]. It is very important for practical biology to evaluate the indices of hemostasis in productive animals. This is due to the possibility of its influence on somatic characteristics [10] and on the activity of many body systems [11]. The study of platelet activity allows the development of age-related norms [12], which will help to detect the developing hemostasiopathy in any condition in a timely manner [13]. Particularly important are these studies for the biology of high-yielding breeds of agricultural animals, since their practical application must ensure the preservation of their productive potential and the prolongation of economic use of animals [14]. Due to the high productivity of the Ayrshire breed cows and the great importance for its maintenance of platelet activity, the need to conduct studies on the physiology of their platelet hemostasis is increasing. The goal of the study is to evaluate the specific features of platelet aggregation in heifers of the Ayrshire breed, which are preparing for insemination.

## MATERIALS AND METHODS

The 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 in March 18, 1986, and confirmed in Strasbourg in June 15, 2006) and approved by the local ethic committee of Federal State Budgetary Educational Institution of Higher Education "Vologda State Dairy Farming Academy by N.V. Vereshchagin" (Record №12 dated December 3, 2015), the local ethic committee of All-Russian SII of Physiology, Biochemistry and Animals' feeding (Record №11, dated December 4, 2015) and the local ethic committee of Russian State Social University (Record №16, dated December 7, 2015).

36 heifers of the Ayrshire breed, which are in optimal conditions of maintenance and are preparing for insemination in the farm of PlemzavodMaysk of the Vologda region (Russia), were examined. Animals were examined three times: at 12 months. age, at 15 months. age and at 18 months. age. Only healthy animals that were planned for insemination were taken under observation.

Blood samples were collected from jugular vein of all the heifers in the morning for studying platelet parameters. Sampling was made into a plastic tube containing 3.8% citrate of sodium dilution in the ratio of blood volumes and citrate of sodium –9:1.

The number of platelets in animals' blood was determined by electron- automatic method on hematological analyzer BC-3000 PLUS (the firm "Shenzhen Mindray Bio-Medical Electronics Co., Ltd.", China).

Platelets' aggregative activity was determined by quantitative method with application of photo-electro-colorimeter KFK-2 (Russia) with such aggregation inductors as ADP, collagen and ristomicin in standard concentrations. Platelets' aggregation was estimated according to the values of summarizing index for platelets' aggregation (SIPA), speed of aggregation (SA) and index of platelets' disaggregation (IPD).

The value of SIPA was found with the help of the formula:

$$SIPA = \frac{E1 - E2}{E1 - E} \times 100\%,$$

where:

E -optical density of rich in platelets plasma in units of optical density;

E1 -optical density of platelet depleted plasma before aggregation in units of optical density;

E2 -optical density of platelet depleted plasma after aggregation in units of optical density.

The value of platelets' aggregation speed was found according to the formula:

$$SA = \frac{E1 - E2}{T},$$

where:

E1-optical density of platelet depleted plasma before aggregation in units of optical density;
E2-optical density of platelet depleted plasma after aggregation in units of optical density;
T-period of time, during which maximal fall of optical density took place, in min.

The index value of platelets' disaggregation was calculated according to the formula

$$IPD = \frac{E3 - E2}{E3} \times 100\%$$

where:

E2 - optical density of platelet depleted plasma after aggregation in units of optical density;
E3 - maximal optical density of platelet depleted plasma, measured in 10 minutes after the addition of an aggregation inductor.

The results were processed by Student's criterion (t). Statistical processing of received information was made with the help of a programme package "Statistics for Windows v. 6.0", "MicrosoftExcel". Differences in data were considered reliable in case of $p<0.05$.

## RESULTS OF THE RESEARCH

The total platelet counts (platelet count, mean platelet count, and thrombocrit) recorded in the study were in the standard range and did not change significantly during the follow-up (Table 1).

**Table 1: Thrombocyte indices in heifers of Ayrshire breed, preparing for insemination**

| Indicators | Age of heifers, n=36, M±m | | |
|---|---|---|---|
| | 12 months | 15 months | 18 months |
| Quantity of platelets, thousand/mcl | 346.8±12.62 | 357.2±9.70 | 362.1±6.25 |
| Average platelet count, fl | 7.2±0.44 | 7.2±0.26 | 7.3±0.19 |
| Thrombote, % | 0.27±0.07 | 0.27±0.05 | 0.27±0.04 |
| Inductor of aggregation ADP | | | |
| SIPA, % | 22.5±0.34 | 23.1±0.86 | 25.6±0.05<br>p<0.05 |
| SA, min | 0.02±0.007 | 0.03±0.006<br>p<0.05 | 0.04±0.007<br>p<0.01 |
| IPD, % | 12.2±0.36 | 13.3±0.46 | 14.2±0.42<br>p<0.05 |
| Inductor of aggregation collagen | | | |
| SIPA, % | 8.1±0.35 | 9.1±0.40 | 10.0±0.26<br>p<0.05 |
| SA, min | 0.07±0.004 | 0.08±0.003 | 0.09±0.007<br>p<0.05 |
| IPD, % | 2.6±0.22 | 3.0±0.24<br>p<0.05 | 3.2±0.31<br>p<0.01 |

ISSN: 0975-8585

| Inductor of aggregation ristomicin | | | |
|---|---|---|---|
| SIPA, % | 8.3±0.45 | 9.5±0.50 | 10.3±0.39<br>p<0.05 |
| SA, min | 0.07±0.003 | 0.08±0.006 | 0.09±0.004<br>p<0.05 |
| IPD, % | 2.2±0.08 | 2.5±0.12 | 2.9±0.11<br>p<0.05 |

Legend: p - reliability of the dynamics of indicators in comparison with the outcome.

The maximum active aggregation of platelets caused ADP. In this process, SIAT with ADP in heifers increased during the process of growth, reaching 25.6±0.05% to its end. In response to collagen, SIAT in animals also gradually increased to 10.0±0.26% during growth. This indicated an increase in the sensitivity of platelets to the inducers of aggregation in the heifers of the Ayrshire breed with the growth of their secretory process from platelets. The activity of platelet aggregation under the action of ristomycin in the observed heifers also increased - the SIAT increased by 24.1% during the observation period.

The rate of appearance of aggregates in Ayrshire heifers in response to ADP during the course of observation was halved. The CA underwent similar dynamics under the action of collagen and ristomycin, which in the heifers at the end of the observation was 0.09±0.007 min and 0.09±0.004 min, respectively.

The registration of the platelet disaggregation index, indicating the stability of the aggregates being formed, made it possible to find out that the most stable were the aggregates that appeared in response to ristomycin, the value of the IDT with it during the growth, increased by 31.8%, reaching the minimum level (2.9±0.11%). Aggregates appearing in response to ADP and collagen during growth were less stable: the IDT for both inducers also increased, reaching 3.2±0.31% with collagen and 14.2±0.42% with ADP.

## DISCUSSION

The current knowledge of the role of hemostasis for the functioning of internal organs makes it possible to consider this system particularly important in maintaining the physiological optimum of the organism [15, 16]. The central place in hemostasis is occupied by platelets, which substantially determine its overall level of activity in various parts of the vascular bed [17,18].

Long-term studies have expanded the understanding of factors that affect platelet aggregation, as well as the preservation of blood in the liquid state. These processes are well studied in many states in humans and animals [19]. At the same time, a large number of aspects of the platelet component of hemostasis in cattle at different ages and under different environmental conditions remain unclear. Also, their pedigree features, in particular in the Ayrshire breed, have not been evaluated, in the course of preparing the heifers for the realization of their productive properties - the period of growing.

During the whole process of growth in the body of heifers, physiologically important anabolic processes occur that increase the capacity of all organs and systems of their organism [20,21]. At the same time, in this period all tissues are very sensitive to the influence of unfavorable environmental factors and need the maximum inflow of blood to them having good liquid properties [22,23].

The observation on the Ayrshire calves on growing has revealed that the number of platelets and their average volume do not exceed the limits of the standard normative values [24,25]. At the same time, the aggregation activity of platelets in them increased during the growth. The most active thrombocytes of heifers reacted to ADP. With increasing age, SIAT calves with this inductor increased. In response to collagen and ristomycin, the SIAT values achieved smaller and comparable values. This indirectly indicated in them a small level of collagen access to the walls of blood vessels for platelets and a low content of von Willebrand factor in the blood [26]. This factor interacts simultaneously with ristomycin and with the glycoproteins GP Ib and GP IIb / IIIa of platelet membranes, providing interaction between aggregating platelets [27,28]. The increase in the rate of aggregation detected in heifers on preservation, in response to all the inductors tested, indicates an increase in the number of corresponding receptors on the platelet membranes [29].

The ability for disaggregation in platelets of heifers during the growth process in response to all agonists did not reach high values. This phenomenon can also be explained by receptor rearrangements of platelet membranes and a slight weakening of intra-platelet activation mechanisms (synthesis of thromboxane, phosphatidic acid and platelet activating factor) [30,31].

It becomes clear that during the growth of the Ayrshire heifers, the receptor mechanisms of adhesion and aggregation processes in platelets are amplified, which is balanced by the growth of their disaggregation ability [32]. The revealed features of platelet activity are very physiologically significant for the growth and development of heifers of the Ayrshire breed in the course of growing, as they largely determine the level of microcirculation in their tissues.

## CONCLUSION

Thrombocyte activity is of great importance for ensuring optimal conditions for microcirculation and metabolism, which strongly determine the productive qualities of the cow. This is an incentive for researching various aspects of the functioning of platelet hemostasis in bovine dairy breeds. In the study, a certain increase in platelet aggregation was found in the Ayrshire breeds, which are in optimal environmental conditions and are preparing for insemination. It was physiologically balanced by some growth in the observed animals of the disaggregation activity of their platelets. The revealed features of platelet aggregation are normative for scientific research and monitoring of the state of animals in farms.

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