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# Physiology Of Platelet Hemostasis In Piglets During The Phase Of Newborns.

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**ABSTRACT**

Thrombocytic hemostasis is very physiologically important component of the hemostasis system. Its functional state largely determines the microcirculation in the capillaries, and hence the rate of metabolic processes throughout the body. It has great physiological significance during the phase of newborn. The success of the development of the organism and the development of its functional activity of productive animals largely depend on the level of functional activity of platelet hemostasis. In this regard, the physiology of research aspects of the activity of platelet hemostasis in newborn animals, including pigs. Agriculture is in great need of these studies, as pigs are important productive animals in many countries around the world. In pigs during the phase of newborns there is a slight increase in the activity of platelet hemostasis. This process is based on changes in receptor and postreceptor mechanisms in platelets. These changes are manifested at the physiological level by increased adhesion, aggregation and secretion. The tendency in the study to increase the activity of platelet hemostasis in piglets during the phase of newborns provides a stable preservation of homeostasis and optimal microcirculation in tissues, adequate to the needs of their actively growing organism.

**Keywords:** piglets, newborn phase, platelets, aggregation, adhesion, secretion.

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## INTRODUCTION

Throughout ontogenesis, the hemostatic system performs a number of physiologically important functions to maintain homeostasis [1,2]. Its normal functioning ensures the preservation of blood in the liquid state during hemocirculation [3,4] and, if necessary, rapid local formation of a thrombus in the area of damage to the vessel wall [5,6,7]. The balanced work of hemostasis ensures minimization of blood loss and preservation of the organism's viability [8,9]. The system of hemostasis regulates the rheological properties of blood [10,11] and thus provides the desired level of tissue trophism and metabolism in the cells [12,13,14] of living organisms.

It is known that the optimal activity of hemostasis supports the optimal viability of animals and the course of their development [15,16]. The great importance of a detailed study of the physiology of hemostasis, including in productive animals, is becoming clear, which will help to intensify their breeding and economic use, taking this knowledge into account. Their use can help in economically beneficial regulation of the functional state of the organism and in the treatment of dysfunctions in animals advancing against unfavorable environmental conditions [17,18,19].

Thrombocytic hemostasis is considered to be the primary and very physiologically significant component of the hemostatic system. Its functional state largely determines the microcirculation in the capillaries, and hence the rate of metabolic processes throughout the body [20,21]. Especially physiologically, this is important during the phase of the newborn. The success of the development of the organism and the development of its functional activity of productive animals depend very much on the level of functional activity of platelet hemostasis [22,23]. In this connection, studies of the activity of platelet hemostasis in newborn animals, including pigs, which are important productive animals in many countries of the world, are of great significance for physiology [24].

In this connection, the goal was to evaluate the activity of the components of platelet hemostasis in piglets during the phase of colostrum nutrition.

## 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).

The study was performed on 38 newborn piglets of the optimal functional status of the large white breed, which were inspected and examined 5 times: for 1 day, for 2 days, for 3 days, for 4 days and 5 days of life. All piglets were obtained from healthy sows with 2-3 farrowing.

After washing and resuspending platelets in them, the cholesterol levels were quantitatively assessed by the enzymatic colorimetry method using the "Vital Diagnosticum" (Russia) kit and the total phospholipids by the phosphorus content in the traditional method.

The state of intramuscular peroxide oxidation of lipids was determined in washed and resuspended platelets by the concentration of malonic dialdehyde in the reduction reaction of thiobarbituric acid and by the content of acyl hydroperoxides. The activity of platelet antioxidant catalase and superoxide dismutase enzymes was assessed by standard methods.

In all piglets, platelet aggregation activity was determined by a visual micromethod using thrombin (0.125 U/ml), ADP ($0.5\times10^{-4}$ M), collagen (1:2 dilution of the base suspension), ristomycin (0.8 mg/ml), adrenaline ($5.0\times10^{-6}$ M) in plasma, standardized by the number of platelets to $200x10^{9}$ platelets [25].

In the examined piglets in intact platelets and platelets subjected to trombone stimulation, the content and level of self-assembly of actin and myosin, as well as the quantitative content of ADP and the intensity of its secretion were evaluated [26]. The results of the study were processed using the Student's test.

## RESULTS

In pigs in the study of their platelets during the phase of the newborn, some of their changes were revealed (Table 1). Thus, during the first 5 days of life in piglets in the composition of platelet membranes, the cholesterol content increased to 0.55±0.010 μmol/$10^9$ platelets, and the total phospholipids decreased to 0.46±0.014 μmol/$10^9$ platelets. This was accompanied by a weakening of the processes of lipid peroxidation in the blood plates. This was judged by a decrease in the concentration of acyl hydroperoxides in platelets of animals by 4.8%, malonic dialdehyde to a level of 0.57±0.013 nmol/$10^9$ platelets. The revealed dynamics of the level of lipid peroxidation in the thrombocytes of pigs during the phase of the newborn has become possible due to the enhancement of their antioxidant protection, assessed by the state of catalase and superoxide dismutase. Their activity in the blood platelets of pigs reached 9260.0±92.0 IU/$10^9$ platelets and 1540.0±11.0 IU/$10^9$ platelets, respectively, by the end of the observation.

The content of actin in intact platelets in physiologically mature piglets on the first day of life was 25.7±0.10% of the total protein in the platelet, tending to increase, reaching 26.6±0.05% on the 5th day of life of the total protein in the platelet (Table 1). The intensity of additional actin formation against the background of platelet aggregation under the influence of a strong inductor in newborn piglets also had a slight upward tendency, remaining low.

In intact platelets of pigs on the first day of life, the level of myosin was 10.2±0.08% of the total protein in the platelet, reaching 11.5±0.12% of the total protein content in the thrombocyte on the 5th day of life. When platelets are aggregated in response to a strong inducer in pigs during the newborn period, a slight increase in this index is established.

All the examined newborn piglets showed a normal number of platelets in the blood. At the same time, on the 1st day of life, the time of onset of platelet aggregation in response to collagen was 36.1±0.09 s. It decreased gradually to 33.1±0.08 s by the end of the neonatal phase (Table 1). A similar acceleration of platelet aggregation in newborn piglets was observed under the influence of ADP - by 8.6% and ristomycin by 7.6%. Somewhat later thrombin aggregation of platelets developed (by the end of the phase in 55.0±0.10 s) and adrenaline aggregation of platelets (by the end of the phase in 98.5±0.18 s).

Another important mechanism for enhancing the functional activity of platelets in piglets during the neonatal phase can be considered as the tendency revealed in the study to increase the content of ADP (by 3.2%) and the activity of its secretion (by 12.8%) when stimulating platelets by an inducer.

## DISCUSSION

The collected facts about hemostasis in piglets are still not complete [28,29]. There is an acute need for science and practice in continuing the study of this vital body system of piglets [30]. Now physiologists give it increasing importance, giving also a big role in its functioning of platelets [31]. Their physiological importance at any age is caused by their connection with the state of blood rheology in the microcirculatory bed and, therefore, with the activity of metabolism in tissues [32,33]. Despite the importance of platelet hemostasis activity and the fine mechanisms providing it, their condition in piglets during the first 5 days of life remains insufficiently studied [34].

The level of functional features of thrombocytes of calves of this breed was also ensured by the tendency characteristic of these animals during the observation period to increase the basal amount of actin and myosin and the tendency to intensify self-assembly of their additional amounts during activation and platelet aggregation in response to an inducer of any strength.

After assessing the facts obtained in the study, it becomes clear that in healthy piglets of the mammary nutrition, the adhesive capacity of the blood platelets tends to increase due to a simultaneous increase in the concentration in their blood of the factor Willebrand (FW), which is a cofactor of platelet adhesion and an

increase in the number of receptors to it - (GPIb) on the surface membranes of the blood platelets [35]. The activation of these adhesion mechanisms in the examined pigs could be judged by the acceleration of aggregation of their platelets in response to ristomycin. The reason for this conclusion is that it is similar in its ability to influence platelets to subendothelial vascular structures [36]. It was previously established that FW is connected by one end of the molecule to collagen, and the other to the platelet through the platelet receptor-glycoprotein Ib, forming the morphological basis of adhesion [37]. It is represented by a chain: collagen - FW - GPIb. In this connection, the acceleration of platelet aggregation with ristomycin gives the right to speak of the development in piglets at the very onset of their ontogenesis of the increase in the number of these receptors on platelet membranes.

The found acceleration of platelet aggregation in response to other inductors also showed an increase between the 1 and 5 days of life in piglets of the number of receptors to them on the surface of the blood platelets, contributing to increased platelet aggregation. Evaluation of the influence of strong and weak aggregation inducers on the process of platelet aggregation in the examined pigs made it possible to speak about the tendency to increase the activity of physiological pathways of platelet activation, which work under normal blood flow conditions [38].

Acceleration of platelet aggregation in newborn piglets was caused by the strengthening work and postretseptonego mechanisms in platelets [39]. This was due to the increase in the content of their membranes cholesterol and tended to decrease in the total phospholipids. This circumstance is able to enhance the expression of their membrane receptors of different kinds and especially fibrinogenemia receptors (GPIIb–IIIa), realizing the connection between platelets during aggregation. The development of these conditions, stimulation of the catalytic properties of the phospholipids of the plasma membrane provides increased generation of active coagulation factors and a large number of active G-proteins [40,41,42].

A very significant hemostatic mechanism of thrombocytes is secretion. It also increased its activity in piglets during the phase of newborns. This was indicated by the increase in accumulation in platelet granules of ADP and an increase in its secretion by thrombin.

## CONCLUSION

In pigs during the phase of newborns there is a slight increase in the activity of platelet hemostasis. This process was based on changes in receptor and postreceptor mechanisms in platelets. These changes are manifested at the physiological level by increased adhesion, aggregation and secretion. The revealed tendency to an increase in the activity of platelet hemostasis in piglets during the phase of neonatal life ensures the preservation of homeostasis and the optimal level of microcirculation in tissues adequate to the needs of their actively growing organism.

**Table 1: Parameters of platelets in newborn piglets**

| Indicators | Newborn phase, n=38, M±m | | | | |
|---|---|---|---|---|---|
| | 1 day of life | 2 day of life | 3 day of life | 4 day of life | 5 day of life |
| Cholesterol cholesterol, µmol /$10^9$ platelets | 0.52±0.016 | 0.53±0.014 | 0.53±0.012 | 0.54±0.015 | 0.55±0.010 |
| Common platelet phospholipids, µmol / $10^9$ platelets | 0.47±0.012 | 0.47±0.008 | 0.46±0.010 | 0.46±0.012 | 0.46±0.014 |
| Acyl hydroperoxide of platelets, $D_{233}$ /$10^9$ platelets | 2.38±0.010 | 2.34±0.012 | 2.31±0.014 | 2.27±0.010 | 2.23±0.009 |
| Malonic dialdehyde of platelets, nmol /$10^9$ platelets | 0.65±0.007 | 0.63±0.009 | 0.60±0.010 | 0.59±0.014 | 0.57±0.13 p<0.05 |
| Platelet catalase, IU/$10^9$ platelets | 9050.0±98.9 | 9080.0±106.5 | 9150.0±127.8 | 9210.0±99.8 | 9260.0±92.0 |

| | | | | | |
|---|---|---|---|---|---|
| Superoxide dismutase of platelets, IU/$10^9$ platelets | 1410.0±14.0 | 1440.0±12.2 | 1470.0±11.6 | 1500.0±9.6 | 1540.0±11.0 p<0.05 |
| The content of actin in intact platelets,% of total protein in the platelet | 25.7±0.10 | 26.0±0.14 | 26.2±0.12 | 26.4±0.09 | 26.6±0.05 |
| The content of actin in platelets against thrombin-aggregation,% of total protein in the platelet | 59.6±0.09 | 60.0±0.08 | 60.3±0.12 | 60.5±0.14 | 60.7±0.12 |
| The content of myosin in intact platelets,% of total protein in the platelet | 10.2±0.08 | 10.7±0.06 | 11.0±0.10 | 11.3±0.09 | 11.5±0.12 p<0.05 |
| Myosin content in thrombocytes against thrombin-aggregation,% of total protein in platelet | 70.0±0.05 | 70.4±0.10 | 70.5±0.08 | 70.7±0.10 | 71.2±0.09 |
| Aggregation of platelets with ADP, s | 45.6±0.12 | 45.0±0.14 | 44.2±0.16 | 43.3±0.09 p<0.05 | 42.0±0.12 p<0.05 |
| Aggregation of platelets with collagen, s | 36.1±0.09 | 35.2±0.08 | 34.2±0.11 | 33.1±0.08 p<0.05 | 32.2±0.10 p<0.05 |
| Aggregation of thrombocytes with thrombin, s | 60.2±0.07 | 58.4±0.09 | 56.3±0.08 p<0.05 | 55.0±0.10 p<0.05 | 54.1±0.06 p<0.01 |
| Aggregation of platelets with ristomycin, s | 47.8±0.12 | 46.8±0.06 | 46.0±0.10 | 45.2±0.07 p<0.05 | 44.4±0.10 p<0.05 |
| Aggregation of platelets with adrenaline, s | 101.5±0.17 | 100.2±0.21 | 99.2±0.16 | 98.5±0.18 | 97.3±0.14 p<0.05 |
| The content of ADP in platelets, mmol /$10^9$ platelets | 3.00±0.09 | 3.03±0.11 | 3.05±0.08 | 3.08±0.10 | 3.12±0.07 |
| The degree of secretion of ADP from platelets on the background of stimulation,% | 31.2±0.32 | 31.9±0.15 | 32.6±0.10 | 33.9±0.12 | 35.2±0.02 p<0.05 |

Legend: p - reliability of the dynamics of newborns taken into account with respect to the onset of the phase.

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