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## Functional Activity Of Vascular Hemostasis In Newborn Calves With Iron Deficiency.

Zavalishina S Yu\*.

Russian State Social University, st. V. Pika, 4, Moscow, Russia, 129226.

### ABSTRACT

Currently, iron deficiency anemia is still a common condition in newborn calves, resulting in inhibition of their growth and development processes and often in death of the animal. The processes of hemostasis sensitively respond to changes in the external environment and the state of the body of the calf, sometimes forming a tendency to thrombosis. The occurrence of anemia in calves inevitably weakens their growing body, affecting the state of the vascular wall. In the anemized animals included in the study, a decrease in the index of antiaggregatory activity of the vascular wall with all inducers and their combinations was noted. The indices of the antiaggregatory activity of the vascular wall with the combination of inductors turned out to be the lowest. In the case of isolated use of aggregation inducers, the lowest index of antiaggregatory activity of the vascular wall with collagen was found due to the weakest inhibition of platelet aggregation with this inducer during venous occlusion. A slightly higher level of the index of antiaggregatory activity of the vascular wall is registered with adrenaline and thrombin. They were superior to the index of anti-aggregation activity of the vascular wall with ADP ( $1.45 \pm 0.12$ ) and ristomycin ( $1.45 \pm 0.06$ ), also reliably yielding to control. In the blood of newborn calves with iron deficiency, depression of the basal level of antithrombin III was noted by 17.6%. This was accompanied by a decrease in their production of antithrombin III by endotheliocytes, which led to a decrease in its stimulated level in blood plasma, forming in the body an obvious deficiency of anticoagulants of vascular origin. It can be considered that in newborn calves with iron deficiency, activated lipid peroxidation in the blood plasma contributes to the weakening of the hemostatic activity of the vascular wall, creating the basis for hemostasiopathy.

**Keywords:** newborn calves, iron deficiency, anemia, vascular wall, hemostasis.

*\*Corresponding author*

## INTRODUCTION

Currently, iron deficiency anemia in newborn calves is still a frequently encountered condition in Russian farms [1,2,3], leading to inhibition of growth [4,5] and animal development [6,7], weakening their resistance [8, 9], the emergence of various infectious diseases [10,11] and often the death of an animal [12,13]. The processes of hemostasis sensitively respond to changes in the external environment and the state of the body of the calf [14,15], sometimes forming a tendency to thrombosis [16,17]. The occurrence of anemia in calves inevitably weakens their growing body, affecting the state of the vascular wall [18,19].

Despite the intensive development of practical biology, anemia in newborn calves retains in modern conditions a fairly high prevalence [20,21] and often a complicated course [22,23]. At the same time, it becomes clear that this condition is accompanied by disturbances in the hemostasis system with insufficient study of developing vascular dysfunctions in the genesis of the iron deficiency state [24,25].

The goal was set in this paper: to assess the state of anti-aggregation, anticoagulant and fibrinolytic activity of the vascular wall in newborn calves with iron deficiency anemia.

## MATERIALS AND METHODS

Research was conducted in strict accordance with ethical principles established by the European Convention 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 38 newborn calves with iron deficiency anemia, having erythropoiesis disorders and signs of a decrease in the amount of iron in their body (serum iron  $12.2 \pm 0.24 \mu\text{mol} / \text{l}$ , siderocytes  $1.8 \pm 0.19\%$ , hemoglobin  $94.9 \pm 0.31 \text{ g/l}$ , erythrocytes  $4.4 \pm 0.16 \times 10^{12} / \text{l}$ ). The control is represented by 29 healthy newborn calves.

The activity of plasma lipid peroxidation (LPO) was assessed by the concentration of thiobarbituric acid-active products using the Agat-Med kit, acyl hydroperoxides (AHP) and the antioxidant potential of the liquid part of blood [4].

The severity of endotheliocytemia was recorded under a microscope. The state of antiaggregation ability of the vessel wall was determined in a sample with a temporary venous occlusion based on a visual micromethod for recording platelet aggregation (AP) with ADP ( $0.5 \times 10^{-4} \text{ M}$ ), collagen (1: 2 dilution of the main suspension), and thrombin (0.125 units/ ml), ristomycin (0.8 mg/ml) and adrenaline ( $5.0 \times 10^{-6} \text{ M}$ ), and also with their combinations - ADP + adrenaline, ADP + collagen and collagen + adrenaline in similar concentrations with standardized platelet count in the investigated plasma  $200 \times 10^9$  platelets before and after temporary venous occlusion with you by calculating the antiaggregation index of the vascular wall (IAAVW) by dividing the time of AP for venous stasis for the time of AP development without it.

The value of the index of the anticoagulant activity of the vessel wall (IACAVW) in the examined calves was calculated by dividing the activity of antithrombin III (AT III) after venous occlusion by its value before it.

To determine the effect of the vascular wall on blood fibrinolytic activity, a method was used to determine the time of stimulated euglobulin lysis before and after temporary venous occlusion, causing tissue plasminogen activator to be released from the vessel wall into the blood and calculating the fibrinolytic activity index of the vascular wall (IFAVW) by dividing the time of euglobulin lysis before occlusion at the time of lysis after it. Statistical processing of the results obtained was carried out using Student's t-test.

## RESEARCH RESULTS AND DISCUSSION

In observable newborn calves with an iron deficiency state, disturbances in the general condition of animals characteristic of anemia were revealed: weakness, lethargy, lack of interest in the surroundings, pallor of the nasal mirror and visible mucous membranes.

In calves with iron deficiency anemia, high activation of free radical oxidation of lipids in the liquid part of blood was noted (AHP  $3.44 \pm 0.25$  D<sub>233</sub>/1 ml, thiobarbituric acid - active products  $5.20 \pm 0.19$   $\mu\text{mol/l}$  with AOA value of  $22.1 \pm 0.27\%$ ). Similar values in the control were  $1.44 \pm 0.09$  D<sub>233</sub>/1 ml,  $3.46 \pm 0.14$   $\mu\text{mol/l}$  and  $33.7 \pm 0.14\%$ , respectively.

For anemizirovanny newborn calves with iron deficiency was characterized by a decrease in the integrity of the endothelial lining due to the weakening of the connection of cells between themselves and subendothelial structures, which was confirmed by a high level of endothelial cytemia -  $5.8 \pm 0.12$  cells/ $\mu\text{l}$  (in control  $1.5 \pm 0.02$  cells/ $\mu\text{l}$ ).

In the anemized animals included in the study, a decrease in the IAAVW was noted with all inducers used and their combinations. The indices of the antiaggregatory activity of the vascular wall with the combination of inductors turned out to be the lowest. With the isolated application of inducers of aggregation, IAAVW was the smallest with collagen in view of the weakest AP inhibition with this inducer during venous occlusion. A slightly higher level of IAAVW is registered with adrenaline and thrombin. They were superior to IAAVW with ADP ( $1.45 \pm 0.12$ ) and ristomycin ( $1.45 \pm 0.06$ ), also reliably yielding to control.

In order to find out in newborn calves with anemia of the anticoagulant activity of the vascular wall in their blood, the level of AT III was evaluated before the test with temporary venous occlusion and after it. It was found that in the blood of newborn calves of the experimental group, AT III levels were depressed by 17.6%. At the same time, they were characterized by a decrease in the production of AT III by endotheliocytes, which led to a decrease in its plasma level, causing a shortage of anticoagulants of vascular origin (IACAVW  $1.23 \pm 0.02$ ).

When elucidating the state of fibrinolytic activity of the vascular wall in anemic newborn calves, the intensity of the production of vascular plasminogen activators was estimated, recorded by the duration of euglobulin lysis [26] before and after the test with metered venous occlusion [27,28]. A significant lengthening of the spontaneous euglobulin lysis time was found in experienced newborn animals, amounting to 32.6%. It was found that the secretion of tissue plasminogen activators, provoked by creating a temporary ischemia of the venous wall in newborn calves with anemia, experienced a significant weakening (IFAVW by 9.4%).

It becomes clear that in newborn calves with iron deficiency anemia, there is a weakening of plasma antioxidant protection with increased LPO with significant depression of antiaggregatory, anticoagulative and fibrinolytic activity of the vascular wall, in many respects ensuring an increase in the activity of hemostatic processes in general, worsening conditions for further growth and development of the animal organism [29,30].

Being one of the most important stages of ontogenesis, the neonatal phase largely determines the adaptation of the organism to environmental conditions [31,32]. It is in the neonatal phase that all organs and systems [33] are formed with an adequate activation of the genetic program of a living being [34] under the influence of environmental factors [35,36]. An important system that binds together the body of a newborn animal is the vascular system [37]. It is multifunctional and through a number of mechanisms is connected with other systems, organs, in turn, affecting the aggregative state of the blood [38]. The activity of the vessel wall, incl. in young productive animals, the level of factors in the blood is maintained that supports the optimal blood rheology and, thus, the homeostasis of the growing organism [39].

Congenital iron deficiency anemia is accompanied by impaired functioning of many organs and systems [40], including the walls of blood vessels with the formation of vasopathy. Depression of antioxidant protection of plasma of newborn calves with anemia against the background of hypoxia developing in them [41] contributes to the activation of LPO in plasma, disrupting the integrity of the endothelium and increasing endotheliocytemia [42]. At the same time, the activation of plasma LPO in newborn calves with iron deficiency also causes a pronounced weakening of the antiaggregation ability of the vascular wall, apparently due to a decrease in the synthesis activity of prostacyclin and NO in it, which lowers the proper level of microcirculation in the tissues necessary for the organism in the beginning of extrauterine the existence of [43].

So, against the background of temporary ischemia of the venous wall in newborn calves with iron deficiency, there was a lack of decrease in the adhesive ability of the blood plates, which was provided by the

weakness of at least two mechanisms. The first is insufficient control of the vascular wall over the density of collagen receptor glycoproteins Ia-IIa and VI on the platelet membrane, which is indirectly established by the non-expression of inhibition of AP with collagen during transient venous ischemia [44]. The second mechanism of depression of control over platelet adhesion in anemic newborn calves is associated with a significant increase in von Willebrand factor production by vascular structures and its intensive binding to its receptors - (GPI B) on the surface of blood plates with insufficient ability of physiological antiplatelet agents secreted by the vessel wall to limit this process [45].

In addition, in conditions of low vascular release of physiological antiaggregants, excessive fixation of strong aggregation agonists — collagen and thrombin to receptors on the platelet membrane — is ensured, enhancing the activity of phospholipase C in them, stimulating the phosphoinositol pathway of activating blood platelets, causing phospholation of the contractile system in them. Under conditions of lack of education in the vessels of PGI<sub>2</sub> and NO, the effect of weak aggregation inducers - ADP and adrenaline on platelet receptors is also excessive, which causes high expression of fibrinogen receptors (GPII-IIIa) and significant phospholipase A<sub>2</sub> activity, regulating release from arachidonic phospholipids, regimen [46].

The weakening of the antiaggregatory activity of the vascular wall in newborn calves with anemia was also confirmed on the model with the combined use of aggregation inducers, showing insufficient production of vascular disaggregating substances, largely simulating the real blood flow conditions in animals in which a number of agonists are simultaneously present [47].

A significant role in the formation of vasopathy with the lack of atrombogenic activity of the vascular wall in anemized calves in the neonatal phase belongs to the weakening of its anticoagulant and fibrinolytic properties [48]. The former are caused by a decrease in production in the intact subendothelium of one of the most powerful physiological anticoagulants - antithrombin III [49-50]. The decrease in the control of the vascular wall of newborn calves with anemia over the blood fibrinolytic activity was provided by depression of the intensity of tissue plasminogen activators synthesis in it [51-53].

## CONCLUSION

In newborn calves with iron deficiency anemia, lipid peroxidation of blood plasma is activated against the background of a weakening of its antioxidant protection and suppression of the hemostatically significant activity of the vascular wall.

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