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Physiological Features Of Anti-aggregational Control Of Blood Vessels Over The Shaped Elements Of Blood In Calves At The Onset Of Ontogenesis.

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ABSTRACT

The success of hemocirculation in capillaries largely determines the overall physiological and biological status of newborn calves. In turn, it largely depends on the characteristics of the aggregation of blood elements and the severity of control over it from the side of the vascular wall. The goal is to establish the severity of antiaggregatory effects of blood vessels on the main blood elements in calves during the phase of newborns. The study was performed on 32 calves of black and motley breed, taken in the study for 1-2 days of life. The examination was carried out during the newborn phase five times - by 1-2, 3-4, 5-6, 7-8 and 9-10 days of life with the use of biochemical, hematological and statistical methods of investigation. During the whole newborn calves showed a tendency to increase the aggregation activity of erythrocytes with a high degree of disaggregating control of the vascular wall above them. This was combined in the observed animals with inactive aggregation of platelets with a tendency to increase their sensitivity to the disaggregating effects of the vascular wall. The low aggregation of neutrophils in calves of colostrum was effectively inhibited by the pronounced antiaggregatory activity of the vessels. Thus, newborn calves have a physiologically justified balance of aggregation of blood elements and disaggregating control over them from the side of the vascular wall.

Keywords: neonatal phase, calves, ontogeny, vascular wall, aggregation, erythrocytes, platelets, neutrophilic leukocytes.

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INTRODUCTION

The need to intensify the production of milk and beef around the world [1] sharply dictates the need for further research in the field of the physiology of cattle during the whole ontogenesis [2,3]. Particular attention in this connection draws blood consisting of uniform elements and plasma [4], continuously moving along the vessels of the animal, ensuring gas exchange, delivery of nutrients and biologically active substances to tissues, and removing toxic substances and wastes from them [5]. The success of hemocirculation, which substantially determines the completeness of the realization of hereditary information [6,7] and the general physiological status of the animal [8,9], is largely related to the level of aggregation of the formed blood elements [10,11] and the severity of control over it from the side of the vascular wall during total ontogeny [12,13].

Due to the high social significance of thrombosis development, the aggregation properties of blood elements [14,15] and vascular disaggregation control over them [16] were actively studied in humans. The most studied in this plan is cardiac pathology [17,18], in particular arterial hypertension, very often combined in the second part of ontogenesis in a person with metabolic disorders [19,20,21]. As a result of these studies, it was possible to find out the mechanisms of data of aggregation-disaggregation processes that are very valuable for physiology, their age dynamics and trace the level of their changes in pathological conditions. In human studies, it has been observed that excessive aggregation of erythrocytes, platelets and leukocytes can disrupt metabolic processes and weaken the body's functional state [22,23]. Also in clinical trials, the possibility of regulating, if necessary, aggregation-disaggregation phenomena directly into the blood was established. In conditions of cardiovascular pathology, the possibility of achieving a balance between them with the help of pharmacological agents [24,25,26] and non-medicamentous effects was demonstrated [27,28,29].

At present, it becomes clear that vascular control of the aggregation properties of blood constituents determines to a large extent the fluid properties of blood and, thus, the optimal microcirculation in the tissues of farm animals throughout the ontogenesis. Their condition is of great biological importance for the processes of growth, development and ensuring maximum disclosure of the productive potential of animals [3].

At the same time, despite the undoubted physiological significance of vascular control over aggregation of the most numerous uniform elements of blood into life-support processes in all species of productive animals in cattle, the data of the study were not previously conducted. Remain not assessed their characteristics at the very beginning stage of ontogenesis - in the phase of newborn, when the liquid properties of blood depend in many ways all the subsequent development of animals. To close this gap in the physiological knowledge system, it was necessary to assess the disaggregation capacity of the vessels in calves during the phase of newborn. The acquisition of this knowledge is not only able to enrich the fundamental science, but will also be useful for practice. clarification of the severity of antiaggregatory vascular disorders in newborn calves in certain diseases [30]. In addition, an assessment of the degree of their dynamics with different approaches to the correction of animal status [31] is possible only if the normative level of the recorded indicators is known. In this regard, the aim of the work is to establish the severity of antiaggregational effects of blood vessels on the main blood elements in calves during the phase of newborns.

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 Kursk Institute of Social Education, branch of Russian State Social University (Record №12 dated December 3, 2015) and the local ethic committee of All-Russian SII of Physiology, Biochemistry and Animals' feeding (Record №11, dated December 4, 2015).

The study was performed on 32 calves of black and motley breed, taken in the study for 1-2 days of life. The examination was carried out during the newborn phase five times - by 1-2, 3-4, 5-6, 7-8 and 9-10 days of life.

The severity of the processes of lipid peroxidation (LPO) in plasma was estimated from the content of thiobarbituric acid-active products by the Agath-Med complex and acyl hydroperoxides [32]. The antioxidant potential of the liquid part of the blood was determined by its antioxidant activity [33].

Vascular control of the aggregation of blood elements was determined by its weakening in plasma obtained after a temporary venous occlusion, by applying a sphygmomanometer cuff for 3 minutes to a limb and injecting a pressure of 10 mm Hg into it higher than systolic.

The severity of aggregation of erythrocytes before and after temporary ischemia of the vessel wall was determined with the help of a light microscope in the Goryaev chamber, recording the number of erythrocyte aggregates, the number of aggregated and non-aggregated erythrocytes [34]. In all calves, by dividing the sum of all red blood cells in aggregates by the amount of this sum against the background of temporary venous occlusion, a vascular control index over the sum of erythrocytes in the aggregate (ICVSEA) was calculated, as a result of dividing the number of aggregates without temporal venous occlusion by their number against a temporary venous occlusion vascular control index over the number of erythrocyte aggregates (ICVQEA) and during the division of the number of free erythrocytes against the background of temporary venous occlusion by the number of free erythrocytes b The value of the vascular control index over the number of free erythrocytes (ICVQFE) was calculated.

Aggregation of platelets (AP) was assessed by visual micrometry of AP evaluation [35] before and after venous occlusion using ADP ($0.5x10^{-4}$ M), collagen (1: 2 dilution of the base suspension), thrombin (0.125ed/ml), ristomycin (0.8 mg/ml), adrenaline ($5.0x10^{-6}$ M), hydrogen peroxide ($7.3x10^{-3}$ M) and combinations of inducers - ADP and adrenaline, ADP and collagen, adrenaline and collagen at the same concentrations in rich platelet plasma with a standardized platelet count of $200x10^{9}$ platelets. The index of antiaggregatory activity of the vascular wall (IAAVW) was revealed by dividing the time of development of AP after venous occlusion for a time without it.

The limiting effect of blood vessels on the process of neutrophil aggregation in plasma obtained after and without cuff application was evaluated by the ability of these cells to aggregate on a photoelectrocolorimeter [34]. Inductors were used lectin wheat germ at a dose of 32 µg/ml, concanavalin A - 32 µg/ml and phytohemagglutinin - 32 µg/ml. All the calves calculated the inhibition index of the vessel wall of neutrophil aggregation (IIVWAN) by dividing the aggregation of neutrophils in a plasma obtained without a cuff by its magnitude in plasma taken with a cuff overlay.

Statistical processing of the results was carried out by Student's t-test.

RESULTS

In the calves examined, the low plasma LPO activity was noted, which tends to decrease during the observation period - the content of acyl hydroperoxides decreased from 1.53±0.26 D_{233}/1 ml to 1.42±0.31 D_{233}/1 ml, thiobarbituric acid -active products from 3.62±0.12 µmol/l to 3.48±0.24 µmol/l. This was accompanied by a trend towards an increase in the actio-oxidative activity of plasma from 32.0±0.42% for the 1-2 days to 33.4±0.28% on the 9-10th day.

During the neonatal phase, the calves tend to increase spontaneous aggregation of erythrocytes, as judged by their tendency to increase the total number of erythrocytes in the aggregate (by 4.7%), the increase in the number of erythrocyte aggregates (by 2.5%) and the slight tendency towards decrease in the number of freely lying red blood cells (by 2.4%) (Table 1).

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Table 1: Aggregation of the basic blood elements in neonatal calves

Registered Parameters	Newborn phase, n=32, M±m				
	1-2 day of life	3-4 day of life	5-6 day of life	7-8 day of life	9-10 day of life
Sum of all the erythrocytes in an aggregate	38.5±0.24	39.2±0.31	39.6±0.39	39.9±0.27	40.3±0.38
Quantity of aggregates	8.0±0.14	8.0±0.18	8.1±0.09	8.1±0.15	8.2±0.19
Quantity of free erythrocytes	253.1±1.34	251.0±1.63	250.1±1.42	248.9±2.08	247.2±1.85
AP with ADP, s	40.8±0.14	40.6±0.16	40.3±0.10	40.0±0.18	39.8±0.19
AP with collagen, s	31.6±0.19	31.4±0.12	31.3±0.11	31.0±0.09	30.8±0.17
AP with thrombin, s	53.9±0.14	53.6±0.12	53.2±0.19	52.8±0.14	52.6±0.20
AP with ristomicin, s	48.6±0.14	48.5±0.19	48.2±0.21	47.8±0.19	47.5±0.23
AP with epinephrine, s	99.8±0.34	99.3±0.29	98.9±0.37	98.4±0.28	97.9±0.31
Aggregation of neutrophils with lectin, %	14.2±0.12	14.2±0.18	14.3±0.16	14.4±0.23	14.6±0.20
Aggregation of neutrophils with concanavalin A, %	13.5±0.13	13.7±0.14	13.9±0.18	14.2±0.09	14.5±0.15
Aggregation of neutrophils with phytogemagglutinin, %	26.2±0.16	26.5±0.18	26.7±0.16	26.9±0.15	27.2±0.22

Note: the reliability of the dynamics of the indicators taken is not obtained.

Against the background of temporary venous occlusion in calves during newborns, the total number of erythrocytes in aggregates in the first 10 days of life decreased by 3.1%, the number of these aggregates decreased by 2.8%, which was accompanied by an increase in the number of free erythrocytes by 1.7% the trend towards an increase in ICVSEA, ICVQEA and ICVQFE (Table 2).

Table 2: Antiaggregational control of the vascular wall over the shaped elements of the blood in newborn calves

Registered parameters	Newborn phase, n=32, M±m				
	1-2 day of life	3-4 day of life	5-6 day of life	7-8 day of life	9-10 day of life
ICVSEA	1.17±0.007	1.19±0.006	1.21±0.004	1.24±0.009	1.27±0.007
ICVQEA	1.11±0.004	1.12±0.006	1.14±0.008	1.16±0.005	1.17±0.003
ICVQFE	1.18±0.012	1.19±0.009	1.20±0.010	1.21±0.006	1.23±0.009
IAAVW with ADP	1.59±0.006	1.60±0.005	1.62±0.007	1.64±0.005	1.65±0.004
IAAVW with collagen	1.56±0.004	1.58±0.005	1.59±0.007	1.60±0.005	1.62±0.008
IAAVW with thrombin	1.50±0.006	1.50±0.004	1.51±0.008	1.52±0.004	1.53±0.006
IAAVW with rhystomicin	1.49±0.005	1.50±0.006	1.50±0.009	1.51±0.007	1.52±0.004
IAAVW with adrenalin	1.62±0.003	1.62±0.006	1.63±0.005	1.64±0.004	1.64±0.008
IAAVW with ADP and adrenalin	1.37±0.008	1.38±0.005	1.38±0.007	1.40±0.002	1.40±0.004
IAAVW with ADP and collagen	1.29±0.007	1.30±0.004	1.30±0.003	1.31±0.004	1.32±0.006

IAAVW with adrenalin and collagen	1.47±0.006	1.48±0.005	1.48±0.007	1.49±0.004	1.50±0.003
IIVWAN with lectin	1.20±0.006	1.22±0.005	1.23±0.006	1.24±0.008	1.26±0.008
IIVWAN with concanavalin A	1.22±0.005	1.24±0.007	1.24±0.008	1.25±0.007	1.26±0.004
IIVWAN with phytogemagglutinin	1.16±0.005	1.17±0.006	1.18±0.007	1.19±0.004	1.20±0.005

All the calves during the neonatal, a tendency to increased platelet aggregation (Table 1). In the sample with temporary venous occlusion of their aggregation the tendency to slow down at. This indicated a tendency to a gradual strengthening observed calves control of the vessel wall on platelet aggregation. It was confirmed they found a trend of increasing values IAAVW have reached the calves to the 9-10 day of life: for the adrenaline of 1.64±0,008 for ADP of 1.65±0,004, for collagen of 1.62±0,008 for thrombin of 1.53±0,006, ristomycin of 1.52±0,004. With the combined use of inducers of index antiaggregatory activity of the vascular wall also had a tendency to increase (Table 2).

During neonatal calves showed a tendency to increase neutrophil aggregation (Table 1). In a sample with temporal venous occlusion, their aggregation had a tendency to weaken with respect to all the inductors tested, which led to a tendency to increase the values of IIVWAN for lectin by 5.0%, for concanavalin A by 3.3%, for phytohemagglutinin by 3.4% (Table 2).

DISCUSSION

Cattle is an important source of milk and meat on the planet. In this regard, a comprehensive study of its physiology is justified, so that it would be possible to use its biological potential to the fullest extent with respect to the level of milk yield [36], obtaining full-fledged offspring [37] and meat production [5]. In modern biological science, an understanding is gradually being formed of the great importance of the rheological properties of blood [38,39], which largely depend on the disaggregation effects on the aggregation properties of blood elements [34].

The study is devoted to assessing the disaggregation properties of the vascular wall in calves during the initial stage of postnatal ontogeny - the phase of newborn. In the animals examined, a stably high antioxidant plasma activity was detected, which ensured effective containment of LPO activity in it. The low intensity of free-radical processes found in newborn calves undoubtedly contributes to the maintenance of the expressed functional activity of endotheliocytes, incl. with respect to their anti-aggregation capabilities [32,35].

Throughout the whole newborn period, a tendency toward an increase in the aggregation activity of erythrocytes was noted, which was effectively suppressed by antiaggregational influences from the walls of the vessels. Obviously, the normal aggregation of erythrocytes in calves in vivo is largely due to the high disaggregating capacity of the vascular wall with simultaneously optimal electronegativity of the erythrocyte surface due to the large number of negatively charged proteins on it [40]. Effective control over the generation of active oxygen species minimizes oxidative damage of these membrane proteins and the optimal plasma protein content [41,42] able to bind red blood cells to each other in already formed aggregates [34]. High control of the vascular wall over aggregation of erythrocytes is based on increased production of prostacyclin and NO in calf newborn calves. Connecting on the surface of red blood cells with their own receptors, they maintain the optimum activity of adenylate cyclase and phosphodiesterase, providing a physiological ratio in their cytoplasm of the amount of cyclic adenosine monophosphate and Ca^{2+}.

The detected upward trend in IAAVW in calves during the first 10 days of life was probably a consequence of a one-time tendency to increase the production of desaggregants in the vascular walls and a tendency to increase the sensitivity of the platelets themselves [4]. The low AP in neonatal calves in response to ristomycin was undoubtedly due to the physiologically minimal production of von Willebrand factor in the vascular endothelium [43]. A sufficiently high duration of AP with combinations of inducers before and after venous occlusion indicated the optimality of vascular-platelet interactions in vivo in the observed animals [44], which created conditions for them to minimize the number of platelet aggregates in their blood.

The revealed low aggregation of neutrophils in the observed calves was undoubtedly due to the large antiaggregation capacity of the vessels and the optimum composition of the glycoprotein receptors of neutrophilic leukocytes and their sensitivity to lectins used as inducers in the study. The weak tendency for the growth of lectin- and concanavalin A-induced neutrophil aggregation in newborn calves was based on a tendency to increase the expression of adhesion receptors and increase in their content of sites containing N-acetyl-D-glucosamine, N-acetyl-neuraminic acid and mannose [34]. The tendency to growth induced by phytohaemagglutinin aggregation was provided by a tendency to increase in their receptors sites of glycoproteins containing bD-galactose. The detected slight propensity to increase neutrophil aggregation was functionally fully balanced in the observed calves by a tendency to increase the values of IIVWAN with all inducers. This should be attributed to the effective containment of neutrophil aggregation in vivo due to the high production in the vessels of calves of this age of prostacyclin and NO.

CONCLUSION

In newborn calves under physiological conditions, there is an easy tendency to increase aggregation of the basic blood elements. This is completely counterbalanced by their tendency to increase the disaggregation capacity of the vascular wall in relation to erythrocytes, platelets and neutrophils.

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