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Physiology Of Vascular Hemostasis In Newborn Calves.

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ABSTRACT

The hemostatic activity of their vascular wall is of particular importance for the vital activity of newborn calves. The optimality of its condition determines the growth, development of the calf during the entire early ontogeny and, ultimately, the degree of development of the productive qualities of the animal during its life. In a study of 29 healthy newborn calves of black and motley and Simmental breeds, the stability of their acyl hydroperoxides and thiobarbituric acid active compounds was found to be stable at the level of 1.59 D_{233}/1 ml and 3.33 µmol/l, respectively, as a result high activity of antioxidant plasma potential. Against the backdrop of low endotheliocythemia in healthy newborn calves, stably high indices of antiaggregatory activity of the vascular wall with all tested inducers and their combinations. Endotheliocytes of newborn calves are characterized by the stability of production of antithrombin III, which provides the required level of anticoagulants of vascular origin in their blood. In this case, the secretion of tissue plasminogen activators revealed during the creation of temporary ischemia of the venous wall in calves during the first 10 days of life had a general tendency to increase.

Keywords: calves, newborn phase, hemostasis, vascular wall, lipid peroxidation.

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INTRODUCTION

From the very onset of ontogeny productive productive animals begin to form their productive qualities [1,2]. Their directed development towards increase is an important task of modern practical biology [3,4]. The success of these actions is extremely important for the modern society, as it must solve an important component of the problem of social stability - ensuring sufficient production of food raw materials for humans [5].

This task can be achieved if modern physiological knowledge is used in practice, especially in the phase of new-born cattle [6,7].

It is recognized that an important element in the homeostasis of the growing calf organism is the hemostatic activity of the vascular wall, which directly determines the formation of many body functions [8,9]. The functioning of the hemostasis as a whole, which determines the fluid properties of the blood and the state of microcirculation in growing organs and tissues, largely depends on the level of antiaggregational, anticoagulant and fibrinolytic capacity of the walls of the vessels of the newborn calf [10,11].

Pervading the entire body of the calf, the vessels are closely connected with all its systems and organs, determining the formation of their functional abilities [12,13]. The most important is the hemostatic activity of the vascular wall in the phase of the newborn, since the optimality of its state determines the growth, development of the calf during the entire early ontogeny and, ultimately, the productive properties of the animal at its end [14]. At the same time, the hemostatic activity of the vascular wall in newborn calves has not been studied yet, its ability to synthesize antiplatelet agents, antithrombin III and tissue plasminogen activators has not been elucidated [15]. In this connection, the present study was planned and conducted.

The goal is to reveal the functional capabilities of the hemostatically significant activity of the vascular wall in healthy calves in the phase of newborn.

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).

All 29 healthy newborn calves of black-motley and Simmental breeds were examined and examined 5 times: 1-2 days, 3-4 days, 5-6 days, 7-8 days and 9-10 days of life.

The complex of examinations consisted of determining the activity of peroxide oxidation of plasma lipids by the content of acyl hydroperoxides, thiobarbituric acid-active products by the Agat-Med company (Russia) and the antioxidant activity of the liquid part of the blood.

The level of endotheliocythemia was determined. The activity of the anti-aggregation capacity of the vessel wall was determined by the traditional method based on the visual micromethod of platelet aggregation registration with ADP (0.5×10^{-4} M), collagen (1:2 dilution of the base suspension), thrombin (0.125 U/ml), ristomycin (0.8 mg/ml) and epinephrine (5.0×10^{-6} M), as well as their combinations ADP+adrenaline, ADP+collagen and collagen+adrenaline at similar concentrations with a standardized platelet count in the plasma of 200×10^{9} platelets before and after temporary venous occlusion with calculation of the index antiaggregatory activity of the vascular wall by dividing the platelet aggregation time in venous stasis for the time of platelet aggregation development without it.

The index of anticoagulant activity of the vessel wall in the calves examined was calculated by dividing the activity of antithrombin III after venous occlusion by its value up to it.

To elucidate the effect of the vascular wall on the fibrinolytic activity of the blood, a method was used to determine the time of stimulated euglobulin lysis before and after a temporary venous occlusion, causing

the release of a tissue activator of plasminogen from the vessel wall into the blood, calculating the fibrinolytic vascular wall index by dividing the euglobulin lysis time to occlusion for the time of lysis after it.

The results of the studies were processed using the Student's test (td).

RESULTS OF THE STUDY

Neonatal calves showed no significant changes in the blood content of the primary products of peroxidation of plasma lipids - acyl hydroperoxides and secondary ones - thiobarbituric acid-active compounds, an average of 1.44±0.09 D_{233}/1 ml and 3.46±0.14 µmol/l for the phase, respectively. The current intensity of peroxidation was possible as a result of the stability of the antioxidant protection of the animal body during the first 10 days of life - their antioxidant plasma potential did not experience significant fluctuations, averaging 33.7±0.14% for the neonatal phase.

For healthy newborn calves, high integrity of the endothelial lining was characteristic due to the expressed communication of cells between themselves and subendothelial structures, which was confirmed by a low level of endotheliocythemia during the first 10 days of life (an average of 1.5±0.02 cells/µl).

In the healthy animals included in the study, there was a persistence during the neonatal phase of index antiaggregatory activity of the vascular wall with all the inducers used and their combinations (Table 1). The highest index antiaggregatory activity of the vascular wall was characteristic of ADP in view of the maximum inhibition of platelet aggregation with this inducer in venous occlusion. A slightly lower level of index antiaggregatory activity of the vascular wall is registered with adrenaline and collagen. They were inferior to index antiaggregatory activity of the vascular wall with thrombin (an average of 1.52±0.05) and ristomycin (an average of 1.51±0.04), while maintaining constancy during the neonatal phase. Indices of aggregation activity of the vascular wall with a combination of inducers, although they were in absolute values below, also did not experience statistically significant fluctuations during the entire phase of the newborn.

Thus, in healthy babies during the neonatal phase, the stability of the antiaggregatory activity of the vascular wall was noted, which can be explained by the constancy of high production in it of substances with antiplatelet activity in the initial phase of early ontogeny.

To elucidate the dynamics of anticoagulant activity of the vascular wall in newborn calves during the first 10 days of life, the blood level of antithrombin III was measured before the sample with temporary venous occlusion and after it (Table 1). It was found that in the blood of healthy newborn calves there is a constant high level of antithrombin III, an average of 99.3 ± 0.16%. At the same time, the stability of production of antithrombin III with endotheliocytes was characteristic for them, in many respects providing an optimal level of adaptation of the organism to the external environment by maintaining the necessary amount of anticoagulants of vascular origin in blood plasma (index of anticoagulant activity of the vessel wall averaged 1.30 ± 0.06).

To determine the state of fibrinolytic activity of the vascular wall in healthy calves, the intensity of development of vascular plasminogen activators was dynamically determined during neonatal period, recorded by the duration of euglobulin lysis before and after the test with dosed venous occlusion (Table 1).

In the examined newborn animals, a clear tendency was found to reduce the time of spontaneous euglobulin lysis, amounting to a total of 3.4%. It was found that the secretion of tissue plasminogen activators, provoked by the creation of temporary ischemia of the venous wall in calves during the neonatal phase, had a general tendency to increase (index of fibrinolytic activity of the vascular wall by 8.1%).

Thus, in healthy newborn calves, against the background of the persistence of antioxidant plasma protection and lipid peroxidation, the stability of anti-aggregation and anticoagulant activity of the vascular wall is noted with a tendency to increase its fibrinolytic effects, largely ensuring the transfer of hemostasis to the level required for the further growth and development of the animal's body.

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Table 1. Vessel hemostasis in newborn calves

Registered parameters	Newborn phase, n=29, M±m					Average value, n=29, 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	
index antiaggregatory activity of the vascular wall with ADP	1.65±0.14	1.67±0.04	1.65±0.09	1.67±0.10	1.75±0.07	1.67±0.12
index antiaggregatory activity of the vascular wall with collagen	1.64±0.03	1.53±0.01	1.57±0.06	1.55±0.02	1.61±0.01	1.58±0.02
index antiaggregatory activity of the vascular wall with thrombin	1.53±0.07	1.50±0.02	1.51±0.09	1.53±0.03	1.52±0.08	1.52±0.05
index antiaggregatory activity of the vascular wall with ristomycin	1.52±0.04	1.51±0.08	1.49±0.02	1.53±0.09	1.51±0.06	1.51±0.04
index antiaggregatory activity of the vascular wall with epinephrine	1.65±0.06	1.64±0.04	1.63±0.02	1.65±0.07	1.64±0.03	1.64±0.07
index antiaggregatory activity of the vascular wall with ADP + epinephrine	1.40±0.09	1.38±0.03	1.40±0.06	1.38±0.01	1.43±0.05	1.40±0.01
index antiaggregatory activity of the vascular wall with ADP +collagen	1.32±0.08	1.29±0.01	1.29±0.06	1.31±0.02	1.36±0.09	1.32±0.01
index antiaggregatory activity of the vascular wall with epinephrine + collagen	1.52±0.15	1.44±0.09	1.42±0.10	1.44±0.08	1.48±0.13	1.45±0.14
antithrombin III, %	96.2±0.11	98.9±0.22	99.6±0.16	99.9±0.15	102.1±0.19	99.3±0.16
antithrombin III after temporary venous occlusion, %	126.0±0.11	130.5±0.17	131.5±0.10	131.9±0.07	132.7±0.16	130.5±0.06
The index of anticoagulant activity of the vascular wall	1.31±0.04	1.32±0.01	1.32±0.07	1.32±0.02	1.30±1.05	1.30±0.06
Time of spontaneous euglobulin lysis, minutes	186.3±0.52	183.6±0.38	181.6±0.49	179.6±0.46	178.9±0.42	182.0±0.39
Time of spontaneous euglobulin lysis after temporary venous occlusion, minutes	253.4±0.07	253.4±0.09	252.4±0.04	249.6±0.11	254.0±0.20	252.6±0.50
The index of fibrinolytic activity of the vascular wall	1.36±0.09	1.38±0.12	1.39±0.11	1.39±0.11	1.42±0.07	1.39±0.10

DISCUSSION

Being one of the most important stages of ontogeny, the phase of newborn in many respects causes adaptation of the organism to environmental conditions [16,17]. It is during the phase of newborns that all organs and systems develop with adequate activation of the genetic program of a living being under the influence of environmental factors [18, 19]. An important system that connects the organism of a newborn animal is the vascular system [20,21]. It is multifunctional and through a number of mechanisms it is connected with other systems, organs, which in turn influence the aggregate state of blood [22,23]. Activity of the vessel wall, including in young animals of productive animals, determines the level in the blood of factors that support the optimal rheology of blood and, thereby, the homeostasis of a growing organism [24,25].

The low level of plasma lipid peroxidation in newborn calves causes a weak alteration of endotheliocytes, contributing to the permanence of the antiaggregatory ability of the vessel wall, apparently due to the high activity of synthesis of prostacyclin and nitric oxide in it, ensuring the proper level of microcirculation in tissues necessary for the body's needs in conditions of the onset of extrauterine existence [26,27].

Against the background of temporary ischemia of the venous wall in healthy newborn calves, there is a marked decrease in the adhesive capacity of the blood platelets, which is provided by two mechanisms [28]. The first is sufficient control from the side 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 constant inhibition of platelet aggregation with collagen during temporary venous ischemia [29]. The second mechanism of strengthening control over platelet adhesion in newborn calves is associated with the constant development of von Willebrand factor by vascular structures and its binding to receptors (GPI) on the surface of blood plates and the pronounced ability of physiological antiplatelet agents actively secreted by the vessel wall in its temporary ischemia to limit this process [30].

Under the conditions of the stability of the exit from the vessels of physiological antiplatelet agents, a constant low level of fixation of strong aggregation agonists, collagen and thrombin, is maintained to receptors on the platelet membrane, inhibiting the activity of phospholipase C, inhibiting the phosphoinositol pathway of platelet activation, weakening the phosphorylation of proteins of the contractile system [31,32]. Under the conditions of constancy of formation in vessels PGI_2 and NO, the action of weak aggregation inducers ADP and adrenaline on platelet receptors is also stable and not expressed, which causes low expression of fibrinogen receptors (GPIIb-IIIa) and a small activity of phospholipase A_2 regulating the yield of arachidonic acid phospholipids [33.34].

Stability during the neonatal phase of antiaggregatory activity of the vascular wall in conditions of combined application of aggregation inducers has shown the sufficiency of development of disaggregating substances of blood vessels, largely simulating real blood flow conditions in animals in which a number of agonists are simultaneously present [35,36].

A significant role in the formation of adequate atrombogenic activity of the vascular wall in calves in the phase of newborn belongs to the constancy of its anticoagulant and to a slight enhancement of fibrinolytic properties [37]. The former are due to the consistently high production of one of the most powerful physiological anticoagulants, antithrombin III, in the intact subendothelium [38]. The pronounced control of the vascular wall of newborn calves over the fibrinolytic activity of the blood is provided by the high intensity of synthesis of plasminogen activators in it, which tends to increase [39]. This is largely due to the consistency of a low level of influence on the vessels of peroxidation of plasma lipids, which controls the level of functioning of endotheliocytes, including synthesis in them and subsequent secretion of plasminogen activators from them [40].

CONCLUSION

The stability of lipid peroxidation in the liquid part of the blood, registered in newborn calves, largely determines the permanence of antiaggregatory and anticoagulant activity of the vessel wall, with a tendency to increase its fibrinolytic ability, ensuring that the newborn calves have the necessary level of control of the vascular wall over the general hemostatic process.

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