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Functional Features Of Hemocoagulation In Newborn Calves Undergoing Acute Hypoxia On The Background Of Corrective Action.

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ABSTRACT

The coagulation system is an important element in maintaining the constancy of the internal environment of the body. Its condition has a special physiological significance at the initial stages of ontogenesis in all productive animals, including cattle. In calves that have undergone acute hypoxia at birth, there has been an increase in the coagulation process, a weakening of the mechanisms for anticoagulation and fibrinolysis. The use of the combination of krezacin and gamavit in the examined animals provided the positive dynamics of all the indicators taken into account. As a result of their use, the level of plasma acylhydroperoxides decreased to the level of the norm. Against the background of the correction in newborn calves who had undergone acute hypoxia, it was possible to fully normalize the activity of all initially activated coagulation factors. The time of general coagulation tests in experienced newborn calves on the background of correction reached the level of control values. As a result of the correction, the activity of antithrombin III and protein C in the observed calves increased, which ensured their normalization. This was accompanied by an increase in the level of plasminogen, ensuring the normalization of the activity of the fibrinolysis system. Thus, for newborn calves who underwent acute hypoxia at birth, activation of coagulation and weakening of the anticoagulant and fibrinolytic mechanisms of plasma are characteristic. The use of a combination of krezacin and gamavit in this category of newborn calves provides for the normalization of plasma coagulation activity and its limiting mechanisms.

Keywords: acute hypoxia, calves, neonatal phase, krezacin, gamavit, blood coagulation.

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INTRODUCTION

The optimal functioning of the body is often disturbed by various negative environmental factors [1-4]. A very frequent point of negative environmental impact is blood [5, 6] and its various systems for maintaining homeostasis and realizing hemostasis [7–10].

The coagulation system is an important element in maintaining the constancy of the internal environment of the body [11]. Its condition has a special physiological significance at the initial stages of ontogenesis in all productive animals, including cattle [12]. The functional properties of the coagulation system largely determine the liquid properties of blood, which provides optimal conditions for the implementation of the hereditary program of growth and development of the animal after birth [13,14].

Until now, in many livestock farms in calves at birth, acute hypoxia is recorded [15,16]. This condition leads to a pronounced decrease in resistance of animals, inhibition of their growth and often cause the death of calves [17]. It is known that this condition can adversely affect many blood parameters and, as it was found out on newborn piglets, causes an increase in the activity of hemocoagulation mechanisms [18]. This facility has previously shown the high efficiency of the combination of krezacin and gamavit. Their use has shown the possibility of normalizing blood coagulation processes while ensuring high animal preservation [19]. At the same time, the state of hemocoagulation in newborn calves after acute hypoxia was found to be insufficient and the approaches to its correction were not developed. Given the high efficiency of the combination of krezacin and gamavit in newborn piglets, it was decided to test its capabilities in optimizing the plasma hemostasis in newborn calves undergoing acute hypoxia at birth.

In this regard, the goal was set in the work: to find out the dynamics of the activity of coagulation hemostasis in newborn calves who underwent acute hypoxia at birth while using krezacin and gamavit.

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).

The study included 33 newborn calves who underwent acute hypoxia at birth (experimental group). The control in the work is presented by the average values of indicators obtained from 35 healthy calves during daily examination during the neonatal phase.

The examination of animals included the assessment of plasma lipid peroxidation activity using the routine method for determining acyl hydroperoxides (AHP) and recording the level of thiobarbituric acid (TBA) -active products using the Agat-Med kit (Russia). All calves were determined by the amount of plasma antioxidant activity according to the method [6]. The functionality of the coagulation system of the blood was determined from each calf under observation by assessing the activity of coagulation factors (I, II, V, VII, VIII, IX, X, XI, XII), the duration of the activated partial thromboplastin time, prothrombin and thrombin time.

Plasma activity of antithrombin III and protein C was determined traditionally.

In calves, the time of spontaneous euglobulin lysis, the activity of plasminogen and α_2-antiplasmin were traditionally estimated.

All observed calves who had acute hypoxia at birth had a correction condition. To do this, for 5 days they were drinking krezacin 4 mg / kg per day, in the morning, and gamavy 0.03 mg/kg was administered intramuscularly once a day, in the morning. Evaluation of the condition of the calves was carried out at the end 5 days after the end of the correction.

Results were processed statistically using Student's t-test (t). Statistical data processing was carried out using the “Statistics for Windows v. 6.0," Microsoft Excel ". Differences in data were considered significant at $p < 0.05$.

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RESULTS OF THE STUDY

In newborn calves who underwent acute hypoxia at birth, an increase in the level of thiobarbituric acid-active products was noted in plasma by 62.9% compared with the control. At the same time, the amount of acylhydroperoxides in the plasma in the experimental group exceeded the level of healthy animals almost 2.3 times. The revealed enhancement of lipid peroxidation in the plasma of experimental calves was possible as a result of a similar weakening of the antioxidant activity of plasma in animals — by 31.2% (Table).

In newborn calves who experienced acute hypoxia at birth, a similar increase in plasma levels of activity I, II, V, VII, VIII, IX, X and XI of coagulation factors was found during normal activity of factor XII. At the same time, in the group of experimental animals, the time of coagulation tests was accelerated: the duration of the activated partial thromboplastin time was reduced by 32.7%, the prothrombin time by 22.1%, the thrombin time by 9.5%.

At the same time, the activity of antithrombin III and protein C in the observed calves of the experimental group was reduced compared with the control by 13.2% and 17.5%, respectively. In both experimental groups, this was accompanied by a similar inhibition of spontaneous euglobulin lysis by an average of 27.9%, a decrease in the activity of plasminogen by an average of 30.9%, and an increase in the activity of α_2 –antiplazmin by an average of 11.6% (table).

The use of a combination of krezacin and gamavit in the examined animals as a correctional effect provided a similar positive dynamics for all indicators taken into account. The levels of acylhydroperoxides and thiobarbituric acid-active compounds in plasma initially increased in test calves decreased as a result of the use of krezacin and gamavit and reached control values due to normalization of plasma antioxidant activity (table).

Against the background of the correction in newborn calves undergoing acute hypoxia, it was possible to completely normalize the activity of all the initially activated enzymatic (I, II, VII, IX, X and IX) and non-enzymatic (V and VIII) coagulation factors while maintaining the XII activity factor (table).

The time of coagulation tests in the observed newborn calves of the experimental group as a result of the correction reached the level of control values. This occurred in these calves as a result of a lengthening of the activated partial thromboplastin time by about 1/3, an increase in the duration of the prothrombin time by about ¼ and an increase in the thrombin time by about 10.1%.

As a result of the correction, the activity of antithrombin III and protein C in the observed calves of the experimental group increased in comparison with the outcome by 13.0% and by 17.7%, which ensured their normalization. The pronounced reduction in the time of spontaneous euglobulin lysis found in this case indicated the normalization of the activity of the fibrinolysis system in all experimental animals. This was largely achieved as a result of the weakening of α_2-antiplasmin in the background of the correction of the initially excessive activity of α_2-antiplasmin and the increase of plasminogen in their blood to the level of healthy animals.

DISCUSSION

Already at the very beginning of ontogenesis, various dysfunctions can often be registered in calves. Very common acute hypoxia. It greatly weakens the body and may cause its death [25]. In the present work, in newborn calves who underwent acute hypoxia at birth, a decrease in plasma antioxidant protection was observed with a comparable increase in the level of acyl hydroperoxides and thiobarbituric acid-active compounds. This inevitably causes them to increase the aggregation of blood cells, the alteration of endotheliocytes and liver structures [26]. These changes can greatly disturb the balance of pro-and anticoagulants in their blood plasma. In the examined animals, this was manifested by the acceleration of coagulation along both coagulation pathways. These disorders increased hypoxia and formed the risk of intraorgan thrombosis.

Excess thrombin formation coming after an episode of acute hypoxia in newborn calves was less restrained by the system of natural anticoagulants weakened in them, incl. antithrombin III and protein C. In addition, dystrophic phenomena in the endothelium, caused by activation of lipid peroxidation and / or hypoxia, largely contributed to the disruption of the binding process of antithrombin III to heparin sulfate and

endothelium glucosaminoglycans, which greatly reduced vascular thromboresistance [10]. The decrease in the activity of protein C detected in the blood of newborn calves after acute hypoxia indicated weakness in these animals of inhibitory control over the activity of factors V and VIII [27]. An increase in the activity of α_2-antiplasmin found in all experimental calves and a decrease in the activity of plasminogen caused a weakening of the fibrinolytic properties of their blood.

Table. Dynamics of biochemical and coagulation parameters in the experimental group of newborn calves treated with krezacin and gamavit

Indicators	Experienced group		Control, n=35
	exodus, n=33	end of observation, n=33	
Acyl hydroperoxide plasma, D_{233}/1 ml	3.02±0.09 p<0.01	1.32±0.14 p_1<0.01	1.33±0.14
Plasma thiobarbituric acid, µmol/l	4.92±0.07 p<0.01	3.06±0.05 p_1<0.01	3.02±0.16
Plasma Antioxidant Potential, %	28.2±0.06 p<0.01	37.2±0.12 p_1<0.01	37.0±0.10
Coagulation factor I, g/l	2.0±0.12 p<0.01	1.5±0.10 p_1<0.01	1.4±0.08
Coagulation factor II, %	67.2±0.22 p<0.05	63.8±0.28 p_1<0.05	64.1±0.15
Coagulation factor V, %	119.5±0.26 p<0.01	89.0±0.46 p_1<0.01	89.2±0.12
Coagulation factor VII, %	78.6±0.37 p<0.05	72.0±0.28 p_1<0.05	72.3±0.08
Coagulation factor VIII, %	132.3±0.45 p<0.01	98.0±0.38 p_1<0.01	97.6±0.12
Coagulation factor IX, %	97.1±0.38 p<0.05	88.3±0.32 p_1<0.05	88.7±0.15
Coagulation factor X, %	65.4±0.32 p<0.05	61.9±0.17 p_1<0.05	62.1±0.14
Coagulation factor XI, %	94.3±0.34 p<0.05	90.3±0.23 p_1<0.05	90.2±0.12
Coagulation factor XII, %	90.9±0.30	90.8±0.18	91.3±0.20
Activated partial thromboplastin time, s	27.2±0.29 p<0.05	36.2±0.22 p_1<0.05	36.1±0.18
Prothrombin time, s	13.1±0.26 p<0.05	16.1±0.22 p_1<0.05	16.0±0.15
Thrombin time, s	15.8±0.22	17.4±0.19	17.3±0.12
Activity antithrombin III in plasma,%	81.3±0.13 p<0.05	91.9±0.20 p_1<0.05	92.0±0.16
Protein C,%	42.8±0.10 p<0.05	50.4±0.11 p_1<0.05	50.3±0.18
Spontaneous time euglobulin lysis, min	241.2±0.49 p<0.01	188.1±0.37 p_1<0.01	188.5±0.38
Plasminogen,%	84.2±0.25 p<0.01	111.2±0.32 p_1<0.01	110.2±0.24
α_2 antiplasmin, %	143.0±0.37 p<0.01	127.9±0.22 p_1<0.01	128.1±0.29

Legend: p - reliability of differences in baseline indicators in the experimental group and in control, p1 - reliability of differences in changes in indicators in the experimental group.

The coagulopathy revealed in the experimental group of animals required an effective correction aimed at optimizing the mechanisms of hemocoagulation. Considering the previously identified high efficiency

of post-hypoxic coagulopathy correction in newborn piglets in combination of gamavit and krezacin, it was decided to evaluate its capabilities in newborn calves who had this condition.

In the study, the use of a combination of krezacin and gamavit was accompanied by the normalization of plasma lipid peroxidation in all observed calves. This has a positive effect on metabolism in the bone marrow, blood vessels and liver. The achieved optimization of the activity of hemocoagulation was possible as a result of the normalization of the activity of all coagulation factors. The revealed dynamics of their activity on the background of krezacin and gamavit in newborn calves after acute hypoxia is the result of positive changes in hepatic metabolism in response to the introduction of these drugs into the body. The results achieved in the group of experimental animals were accompanied by an increase in plasma anticoagulant capacity. The increase to the level of the initial low activity of antithrombin III optimized the generation of thrombin, and also controlled the amount of active VII, IX, X, XI and XII coagulation factors in the plasma. The applied correction also normalized in the blood of all experimental calves the activity of protein C, ensuring in them the optimal plasma inhibitory control over V and VIII clotting factors. At the same time, in the observed newborn calves on the background of krezacin and gamavit, it was possible to achieve an increase in the intensity of plasminogen synthesis with the suppression of the excess anti-plasmin activity of their plasma, thereby normalizing the fibrinolysis process [11]. The evolving situation has provided full normalization of the entire hemocoagulation system, the optimum liquid properties of blood and functionally favorable conditions for the realization of their genetic potential.

CONCLUSION

In newborn calves who underwent acute hypoxia at birth, there is an excessive increase in plasma coagulation activity and a decrease in its anticoagulant and fibrinolytic parameters. As a result of the use of a combination of krezacin and gamavit in these newborn calves, plasma coagulation activity and its anticoagulation and fibrinolysis mechanisms were normalized.

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