

Conference Paper

State of the Hemostatic System in the Newborn Calves with Diverse Birth Weights

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Abstract

The studies on the peculiarities of hemostatic indices in calves (n=250) of Holstein-Friesian breed with diverse birth weights were conducted under the conditions of a dairy farm with the aid of generally accepted clinical, instrumental and laboratory methods. The research has demonstrated that in comparison with the adults the newborns weighing from 36.5 to 29 kg revealed a diversity of hemostatic indices but generally there was a functionally acceptable balance of its links without a tendency to coagulopathy or thrombosis. Fetal growth restriction in cattle with low birth weight has a significant effect on the hemostatic system. Insufficient weight of more than 7 % provokes hypercoagulability syndrome which is caused by increased levels of adrenalin, toxic products of impaired metabolism and destruction of erythrocyte membranes. The newborns with lower weight demonstrated signs of primary coagulopathy conditioned by functional inferiority of the organs in which the synthesis of coagulation factors occurred that led to the development of disseminated intravascular coagulation. The revealed dependence of the hemostatic disorders evidence on the severity of weight insufficiency indicates coagulopathy, emerging in epigenetics of the main pathology after primary damage and conditioning the severity of the newborns' state, as one of the main mechanisms of hypotrophy pathogenesis.

Keywords: cattle, newborn calves, hypotrophy, hemostasis, coagulopathy, disseminated intravascular coagulation (DIC).

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1. Introduction

In order to maintain hemostasis, the physiological state of the hemostatic system is of great importance, the main task of which is to preserve the liquid state of the blood in the bloodstream and stop bleeding from damaged vessels by thrombosis. Structurally the organs and tissues involved in hemostasis complete their development by the birth of a fetus. However, they significantly differ from the state of adults in functional terms [1, 2]. These peculiarities of coagulation potential in newborns condition and its high variability during the intra- and neonatal periods create the threat of thrombotic and hemorrhagic complications [3, 4]. Meanwhile, the risk of coagulopathy increases in the presence of perinatal pathology, also under weight insufficiency [5, 6].

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Inborn (antenatal) hypotrophy of calves is one of the most common forms of perinatal pathology in cattle [7]. However, scientific literature represents insufficient data on the effectiveness of the coagulation system functioning in calves with intrauterine growth restriction [4, 8, 9]. In addition, it is known that the severity of pathological changes depends on the strength of the damaging factor influence [10]. However, there is no information on the cause-effect relation between the weight insufficiency of the newborns and the severity of hemostatic disorders, which limits the objective risk assessment and tactics of coagulopathy correction in the newborns with perinatal pathology. Therefore, we conducted the studies on the peculiarities of the hemostatic system in the newborn calves with diverse body weights in order to specify patterns of cause-effect relationships between the severity of hypotrophy and coagulopathy.

2. Materials and Methods

2.1. Animals

This study was approved by the Ethics Committee of FGBNU "VNIVIPFiT" (FSBSI "ARVRIPP&T) and all the rules of humane treatment of animals were observed. The experiment was conducted on agro-industrial complex specialized in milk production, where animals of Holstein breed were kept. The object of it were the calves (n=250), obtained in uncomplicated calving, with no signs of malformation, birth trauma and intranatal asphyxia. The calves were licked by the mother in the first minutes after birth, and then they were placed into a thermal cage, where they were artificially dried at air temperature of 38.5–40.0 °C. At the age of 60 minutes, the samples of their blood were taken and they were weighed, and then they were given 2 liters of colostrum in a nipple drinker. The newborns were kept in an individual cage of preventorium with a relative humidity ranged from 60 to 64 %, and the air temperature of 18–25 °C.

2.2. Clinical examination

The evaluation of calves' clinical state was performed by standard methods. The calves were weighed with Momert 6681 (Hungary), and the results thus obtained became the basis for the formation of 6 experimental groups of 32–35 calves with different body weights: 36.5–29.0; 28.9–28.0; 27.9–27.0; 26.9–26.0; 25.9–25.0; 24.9–24.0 kg

2.3. Laboratory research

Blood samples were taken from the jugular vein into 4 plastic tubes: No. 1 and 1a -- to determine the clotting time of the blood; No. 2 -- with a 3.8 % solution of 5.5-aqueous triple sodium citrate (0.1 ml/ml of blood) to obtain blood plasma, and No. 3 -- with a solution of lithium heparin (17 IU/ml of blood) to maintain its intact state. Hematocrit and platelet count in the obtained samples were determined using an ABX Micros 60 CT/OT hematology counter (France). To evaluate the hemostatic system, the blood coagulation time was studied using a unified method with native blood and Lee-White method, Quick's prothrombin time [11], fibrinogen level was determined by a unified gravimetric method, fibrinase activity -- by a unified accelerated method, and recalcification time and plasma tolerance to heparin -- according to Alekhin Yu.N. [12].

The concentration of soluble fibrin-monomer complexes (SFMC) was estimated using a commercial reagent kit (Scientific Production Association "Renam", Russia). In addition, the sorption capacity of erythrocytes (SCE) was determined according to A.A. Togaybayev et al. [13], extra-erythrocyte hemoglobin (EEH) -- by the hemoglobin cyanide method [14], and the state of membrane-receptor complexes on the surface of erythrocyte membranes was evaluated using a method based on a comparison of the hemolysis degree of erythrocytes in intact samples and after the entry of modifiers (adrenaline and adrenaline blocker) with the subsequent calculation of the modification coefficient (MC) [12].

2.4. Statistical analysis

Mathematical and statistical processing of the obtained data was performed using the program SPSS version 22 (IBM Corp, Version 22.0, Armonk, NY, USA, 2013). The calculation of arithmetic average, its error ($M \pm m$) and the reliability of the difference (p) according to Student criterion were performed.

3. Results

The data of table No. 1 shows that in comparison with the control (36.5--29.0 kg), the calves weighing 28.9--28.0 kg demonstrated no significant changes of the studied indices (table 1). The animals with the body weight of 27.9--27.0 kg demonstrated a decrease of coagulation time by 5.3 % and recalcification decrease by 27.8 %, also an

increased content of EEH by 15.2 %, fibrinogen -- by 22.2 % and high MC level exceeding the control group index by 29 %.

The calves weighing 26.9--26.0 kg demonstrated an increase of blood MC level by 22 %, EEH -- by 63 %, fibrinogen -- by 51.9 %. At the same time, these animals had a reduced blood coagulation time by 10.5 %, recalcification time -- by 36.4 % and plasma tolerance to heparin -- by 6.8 %, but increased prothrombin time by 46.5 %.

The animals weighing 25.9--25.0 kg demonstrated a decrease in platelet count by 12.3 % and plasma tolerance to heparin by 15.6 %, and the rates of blood coagulation, the amount of fibrinogen and soluble fibrin-monomer complexes were increased by 11.4, 23.4 and 21.4 %, respectively (Table 2). In addition, increased levels of extra-erythrocyte hemoglobin (by 2.7 times), MC and SCE by 6.5 and 14.1 % were also registered.

TABLE 1: Blood indices of the newborns with body weight from 27.0 to 36.0 kg.

Indices	Body weight, kg		
	36.5--29.0	28.9--28.0	27.9--27.0
Number, heads	35	35	32
EEH, g/L	0.44±0.032	0.42±0.029	0.42±0.027
MC	1.23±0.005	1.28±0.007	1.59±0.007 ³
SCE, %	39.0±2.33	38.7±1.80	39.3±2.06
Platelets, 10 ⁹ /L	377.0±5.80	367.0±4.35	367.0±6.90
Coagulation time, min	4.05±0.122	3.97±0.118	3.70±0.106 ³
Fibrinogen, g/L	2.86±0.017	2.86±0.024	3.25±0.020 ³
Prothrombin time, s.	23.0±0.21	23.0±0.27	24.0±0.25 ³
Recalcification time, s.	140.0±3.00	141.0±2.70 ¹	110.0±2.02 ³
Plasma tolerance to heparin, s.	189.6±3.00	190.0±2.88	165.0±2.00
Fibrinase activity, s.	111.0±2.03	111.1±1.77	111.0±1.74
SFMC, *10 ⁻² g/L	3.50±0.025	3.75±0.025	3.50±0.022

Note: Distinction with the data of the calves of the first weight range, statistically significant: «1» -- P≤0.05; «2» -- P≤0.01; «3» -- P≤0.001.

The animals weighing 24.9--24.0 kg in the study of hemostatic system demonstrated a decrease in the number of platelets by 24.3 % and the level of fibrinogen -- by 26.6 %, but soluble fibrin-monomer complexes were increased by 57.1 %, however, there were no significant changes in other coagulation indices. At the same time, we also registered a decrease of MC by 30.9 % and an increase of sorption capacity of erythrocytes and extra-erythrocyte hemoglobin by 2.8 times.

TABLE 2: Blood indices of the newborns with body weight from 24.0 to 26.9 kg.

Indices	Body weight, kg		
	26.9--26.0	25.9--25.0	24.9--24.0
Number, heads	33	32	35
EEH, r/l	0.58±0.052 ²	1.17±0.111 ³	1.25±0.101 ³
MC	1.60±0.003 ³	1.31±0.003 ³	0.85±0.005 ³
SCE, %	40.9±2.50	44.5±2.13	45.1±1.97 ³
Platelets, 10 ⁹ /L	355.0±5.80	330.5±4.96	285.8±6.13 ³
Coagulation time, min	3.50±0.042 ³	4.51±0.037 ³	6.84±0.025 ³
Fibrinogen, g/L	4.00±0.011 ³	3.53±0.010 ³	2.10±0.100 ¹
Prothrombin time, s.	33.7±0.35 ³	23.3±0.21	22.0±0.55
Recalcification time, s.	95.5±2.07 ³	135.0±3.90 ¹	125.0±6.87 ³
Plasma tolerance to heparin, s.	175.0±2.25 ³	160.0±2.08 ³	166.6±5.00 ³
Fibrinase activity, s.	120.5±3.08	122.7±2.14	117.0±5.20
SFMC, *10 ⁻² r/l	3.50±0.025	4.25±0.020	5.50±0.028

Note: Distinction with the data of the calves of the first weight range (36,5029,0 kg), statistically significant: «¹»- P≤0.05; «²» -- P≤0.01; «³»- P≤0.001.

4. Discussion

The obtained data showed that healthy calves in 60 minutes after birth, despite the presence of quantitative and qualitative differences in almost all indices of hemostatic system from those in adults [15, 16], generally had a functionally acceptable balance of hemostatic units without a tendency to coagulopathy or thrombosis. Antenatal growth restriction of fetuses with a decrease of their body weight at birth has a significant effect on hemostatic system, which is determined by a combination of impaired organogenesis and metabolism. The hemostatic system changes during the entire time of fetal growth [17], therefore, any malfunction of the development of the vascular system or organs in fetuses may cause primary functional insufficiency of hemostasis [6, 18]. However, the main component of hypotrophy is an imbalance of metabolism with a decrease in its efficiency and the formation of a relatively large number of metabolites with toxic properties [7], which can cause coagulopathy [19, 20]. The presence and combination of the mentioned mechanisms of hemostatic disorders in calves with hypotrophy depends on the severity of body mass deficiency. Thus, the decrease of body weight in the newborns by 1 kg from the lower border of the reference interval of the weight of the Holstein-Friesian newborns (29.0 kg) did not have a significant effect on the studied indices of hemostatic system. With a weight deficit of 3.8--7 % (27.9--27.0 kg), a decrease

in coagulation time and recalcification of plasma citrate were detected, which indicated the activation of prothrombinase formation, and there was also an acceleration of fibrin formation which was indicated by an excess of fibrinogen [11]. One of the reasons for the marked activation of the 1st and 3rd blood coagulation phases is an increase of plasma adrenaline concentration [21], as indicated by an increase of adrenoreceptors activity on the erythrocyte membranes. Increased body mass deficiency up to 10 % (26.9--26.0 kg) is accompanied by an increase of adrenaline excess and hemostasis imbalance severity. Thus, in addition to the intensification of prothrombinase and fibrin formation, these animals demonstrate an inhibition of synthesis of blood-coagulation factors that are included in the "prothrombin complex", which as a result inhibits the conversion of prothrombin to thrombin. A decrease of plasma tolerance to heparin indicates a weakening of the anticoagulant activity [11]. The combination of fibrinogen deficiency and the factors of "prothrombin complex" indicates a functional deficiency of the protein-synthesizing liver function [22, 23]. The newborns with a body mass deficiency from 10 to 14 % (25.9--25.0 kg) demonstrate a decrease of adrenoreceptors activity on the erythrocyte membranes that suggests a decrease of the stress response severity, which is probably related to the relatively low level of adaptation potential in these animals. Despite the weakening of adrenaline initiating effect on hemostasis, the imbalance among the components of hemostasis is preserved and intensified with hypercoagulation preservation, but the platelet and anticoagulant link is weakened. An elevated SCE level suggests that endotoxins are one of the causes of coagulopathy. In addition to the direct effect of toxic metabolites on blood coagulation [24], the permeability of erythrocyte membranes increases and the concentration of extra-erythrocyte hemoglobin rises, which itself is an activator of the coagulation cascade [25], and the damage of erythrocyte membranes structure leads to their death and aggregation, which initiates thrombus formation [11]. The revealed hypercoagulation against the background of a low level of anticoagulative activity caused an increase of the number of fibrin and fibrinogen dehydration products, which indicated the start of the cascade of disseminated intravascular coagulation [26]. The most severe body weight deficit of the newborns in our experiment was 14--17 % that was used for hemostatic profile observation, characterized by a low level of platelets and fibrinogen, but an increased amount of soluble fibrin monomer complexes, which indicated the presence of thrombohaemorrhagic syndrome in animals [11]. Probably, this syndrome had already occurred in the fetus, but there was no doubt that it had a primary failure of the hemostatic system due to dysorganogenesis, which caused an inadequate reaction

during the period of acute adaptation after birth and initiation of the disseminated intravascular coagulation cascade.

5. Conclusion

Fetal growth restriction in cattle with a decrease of the newborns' body weight at birth has a significant effect on the hemostatic system. So, the animals weighing less than 27 kg demonstrated a hypercoagulative syndrome, the causes of which were an increased level of adrenaline, toxic products of impaired metabolism and destruction of erythrocyte membranes. This situation indicates that the fetal body response to the factors inhibiting its development is similar to the metabolic response to stress. With increasing body mass deficiency, signs of primary coagulopathy appear, due to the functional inadequacy of the organs in which the synthesis of coagulation factors occurs. The combination of hypercoagulation and insufficient synthesis of coagulation factors leads to the development of disseminated intravascular coagulation syndrome. The revealed dependence of hemostatic disorders severity on the severity of body mass deficiency indicates coagulopathy as one of the leading mechanisms of the pathogenesis of hypotrophy and arises in the dynamics of the main pathology development following primary damage (failure of antenatal development) and determining the severity of the newborn's state.

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Conflict of Interest

The authors have no conflict of interest to declare.

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