Physiological features of hemostasis in newborn calves receiving ferroglukin, fosprenil and hamavit, for iron deficiency

ABSTRACT
Newborn calves still often suffer from iron deficiency. This state damages their growth and development - to some extent, due to the development of pathological changes in hemostasis. Thereby, both veterinary science and cattle physiology give great scientific and practical significance to the search of approaches to effective correction of new-born calves’ hemostasis pathology connected with iron deficiency. It seemed to be perspective to evaluate the influence of ferroglukin, traditionally applied at iron deficiency, in combination with metabolism stimulators (fosprenil and hamavit) on new-born calves’ indices of hemostasis system. During our study it was established that newborn calves with iron deficiency were also characterized by decreased plasma antioxidant protect-ability, intensity of lipids’ peroxidation processes, increase of platelets’ hemostatic activity and bone coagulation system along with the decrease of vascular wall’s ability to bind it. In our study we found that combination of ferroglukin, fosprenil and hamavit given to new-born calves with iron deficiency showed improved plasma antioxidant and lipid peroxidation activity. Normalization of platelet activity, positive dynamics of hemostasis vascular and plasma components were also observed. Iron deficiency of new-born calves can be considered as the model of hemostasis abnormality. With its help we could try different means and their combinations to cure the pathology of hemostasis. The obtained results allowed us to consider the usage of fosprenil and hamavit combination on the background of ferroglukin to be sufficient for reducing the pathology of hemostasis in newborn calves with iron deficiency.

Keywords: new-born calves; iron deficiency; hemostasis system; ferroglukin; fosprenil; hamavit.

1. INTRODUCTION
The aims of durable and well-planned researches in the field of cattle physiology are conducted to accelerate the processes of its growth, to increase its productivity [1], to work out the ways of modern prophylaxis of different abnormalities and to create approaches for curing the developed pathology [2]. It is very important to accumulate knowledge about different sides of calves’ physiological processes at the very beginning of their ontogenesis [3] because of special significance of the phase of birth. It lays the foundation for milk and meat productivity of cattle. At the present moment it is assumed that the leading role in support of hemostasis during the whole ontogenesis belongs to blood and its hemostatic mechanisms influencing heavily the way of individual development [4,5] with the help of hemocirculation processes. So, activity changes of hemostasis processes change activity of hemocirculation in tissues and organs, and, thus, influence the common state of a body [6]. In previous researches it was found that quick increase of hemostasis components’ activity able to lead to microcirculation abnormalities [7] could take place in homeostasis deviations, especially in case of a young organism. Basic studies in this field were conducted on a human being [8,9,10]. Basing on their results we managed to make a conception of age-specific dynamics of hemostasis components’ activity [11,12], its most vulnerable mechanisms and the potential of different variants of impact on a body aiming at haemostatic processes’ optimization. Because of great social significance of thrombosis development in case of cordial pathology [13,14] many researchers’ attention is still devoted to haemostatic changes in given patients [15,16,17]. They investigated different aspects of hemostasiopathy pathogenesis of cordial diseases. As a result, the possibility of its correction was found. It can be done not only with the help of medicines [18,19], but also with the help of traditionally applied means [20,21]. It has great significance for biological investigations as it allows minimizing application of medicines.

Taking all this into account, we have large scientific and practical interest to the estimation of hemostasis state of new-born calves in conditions of different dysfunctions. We are also eager to find some ways of a body state correction which can positively influence the manifestations of hemostasiopathy. We thought to be perspective to take iron deficiency as a model of hemostasis abnormality, as it was often found in new-born calves, and tried to work out an effective way of correction of all the hemostasis system components. The given model seemed to be justified as it was to result in the decrease of hemoglobin content in blood and activity lowering of iron-bearing enzymes which suppressed protein synthesis and activity of cellular functions [22]. Furthermore, iron deficiency was estimated as a state accompanied by changes in the whole body’s activity. It also led to abnormalities in all the components of hemostasis system.
Moreover, the investigations, aimed at curing hemostasiopathy in calves with iron deficiency, had a great scientific and practical significance. Worked out at the given stage variants of evident decrease of hemostasis abnormalities can serve the basis for the following creation of correction complexes able to be effective in the field of hemostasiopathy reduction in new-born calves at many diseases. Clearing out the impact of combination of iron-bearing substance [23], fosprenil [24] and hamavit [25] (which showed earlier their high biological activity as far as separate hemostasis components are concerned) has serious perspectives for reducing iron deficiency. In addition to this, the purpose of our research was to find the evident ability of ferroglukin, fosprenil and hamavit combination to correct hemostasis system activity of new-born calves with iron deficiency.

2. MATERIALS AND METHODS
2.1 Materials
The study used 34 newborn calves with manifestations of erythrogenesis and decrease of iron content in their bodies (serum iron 12.3±0.10umol/l, siderocytes 1.6±0.05%, hemoglobin 95.0±0.29 g/l, erythrocytes 4.1±0.13x10¹²/l). The control group consisted of 29 healthy newborn calves.

All the investigations in the present study were conducted in full correspondence with ethical norms and recommendations on humanization of work with laboratory animals containing “The European Convent on the protection of vertebrate animals used for experiments or in other scientific purposes” (Strasbourg, 1986).

2.2 Methods
The state of lipids' peroxidation (LPO) in animals' plasma was found out according to the quantity of thiobarbituric acid—active products in it with the help of a set produced by the firm “Agat-Med” (Russia) and acylhydroperoxides with the account of antioxidant activity level of the liquid part of blood [26]. Platelets’ number in calves’ blood was found by their calculation in Gorjaev’s box. Platelets’ aggregation was registered by visual micromethod [10] with some inductors: with ADP (0.5x10⁻⁴ M), with thrombin (0.125un/ml), with collagen (dilution 1:2 of the main suspension), with ristomicin (0.8 mg/ml), with epinephrine (5x10⁻⁶ M) in plasma with standardized quantity of platelets in it (200x10⁷ tr.).

Disaggregative abilities of vascular wall were determined with the help of a test with temporal venous occlusion on the basis of visual micromethod of platelet aggregation registration [10] with all the applied inductors. We calculated the value of vascular wall disaggregative activity index (VWDAI). It was made by dividing the platelets’ aggregation period on the background of venous deadlock on the time of platelets’ aggregation appearance without it. The index value of vascular wall anticoagulation activity of examined calves was also calculated by dividing antithrombin III activity after venous occlusion on its value before it [27]. Vascular control over fibrinolytic blood activity was found out by calculating the index value of vascular wall fibrinolytic activity. We divided the period of euglobulinlysis before occlusion on lysis period after it [27].

The state of plasma hemostasis was evaluated according to duration of activated partial thromboplastin period (APTP), prothrombinic period and thrombin period with the help of generally applied methods [27].

The correction of iron deficiency state of new-born calves was made by applying ferroglukin intramuscularly, once a day from the calculation of 15mg of iron on 1kg of body mass, fosprenil-0.1mg/kg intramuscularly in the morning as liquid feeding for 6 days and hamavit—0.1ml/kg intramuscularly as liquid feeding for 6 days beginning simultaneously with ferroglukin application. The evaluation of the animals’ health was made twice—at their birth and on the 7th day of life. Because of the absence of reliable differences between the results of both studies, control values of each index were presented by one figure—a simple average between them. Examination of calves with iron deficiency was conducted twice—at their birth and on the next day after correction finish (the 7th day of life). The results were processed by Student's criterion (т). Statistical processing of received information was made with the help of a programme package “Statistics for Windows v. 6.0”, “MicrosoftExcel”. Differences in data were considered reliable in case of p<0.05.

RESULTS AND DISCUSSION
The examined newborn calves with iron deficiency had usual for the given state weakness, limpness, absence of interest to the environment, paleness of rhino scope and slime layers. These animals also had increased LPO activity in plasma (acylhydroperoxide 3.42±0.012 D₂₃₃/1ml, thiobarbituric acid-active products 5.19±0.019umol/l at value depression of antioxidant activity of blood liquid part 22.0±0.23%). The control values of these indices were equal to 1.45±0.010 D₂₃₃/1 ml, 3.46±0.012 umol/l and 33.7±0.15%, respectively.
Platelets’ quantity in newborn calves’ blood was equal to norms. Besides, platelets’ aggregation of animals with iron deficiency turned out to be reliably increased (table). Their earliest platelets’ aggregation appeared in response to collagen (19.8±0.15s), a bit later it developed with ADP and with ristomicin, then later in response to thrombin (37.9±0.21s). The latest platelets’ aggregation of calves with iron deficiency appeared under epinephrine impact (68.2±0.25s).

Newborn calves with iron deficiency had VWDAl decrease in relation to all the applied inductors (table). The lowest VWDAl value belonged to collagen, a bit higher VWDAl value was for thrombin, yet higher VWDAl value was with ristomicin, ADP and epinephrine.The examined animals with iron deficiency had decrease of vascular wall anticoagulant ability. It was found according to the decrease of the index value of anticoagulant activity of a vascular wall. Fibrinolytic features of these animals’ vessels were also weakened – the index of vascular wall fibrinolytic activity was decreased on 13.0%.

Newborn calves with iron deficiency were also characterized by APTP (42.3%) and prothrombin period (42.6%) increase accompanied by some intensity of thrombin period (8.7%).

Conducted correction of calves’ state provided them with iron deficiency improvement of the common state and increase of their serum iron level to the control values (29.3±0.12 umol/l). On the background of application of ferroglukin, fosprenil and hamavit combination examined calves had evident decrease of acylhydroperoxides (2.16±0.010 D_{233}/1 ml, p<0.01) and thiobarbituric acid-active products (4.12±0.014umol/l, p<0.01) in plasma content at the increase of antioxidant activity to 29.1±0.09% (p<0.01).

The process of correction of animals’ iron deficiency was accompanied by invariability of platelets’ quantity in their blood and some slowdown of platelets’ aggregation. Besides, animals’ platelets most actively responded to aggregation with collagen, ADP and ristomicin, less actively – with thrombin and adrenaline addition into plasma (table).

The examined calves after conducted application had evident VWDAl increase in relation to all the applied inductors (table). The minimum value was VWDAl value with thrombin. Other VWDAl values were a bit higher and had a tendency to approaching the control values. Newborn calves with iron deficiency received iron preparation in combination with metabolically active means and had a tendency to the index increase of vascular wall anticoagulant activity and the index rise of vascular wall fibrinolytic activity on 9.5%.

Thanks to complex application we reached APTP slowdown on 42.6% at simultaneous decrease of prothrombin period on 42.6%. It allowed them to get normalized. Besides, the value of thrombin period, defining the activity of fibrinogen transition into fibrin, increased on 8.9% and reached the control level.

Realization of genetically determined growth and development processes of living beings takes place under constant influence of numerous external and internal factors of environment [28]. Physiological peculiarities of their influence are mostly expressed by the optimum of living beings’ blood content [29]. It is especially essential for the activity of hemostasis system components [30]. Besides, any abnormalities in a body are accompanied by negative dynamics of hematological indices [31,32] including parameters of hemostasis system [33,34]. It became clear, that on the basis of hemostasiopathy development we found not only iron deficiency but also depression of plasma antioxidant protection. Previous research showed that it caused LPO activation in it. Increase of peroxidation in plasma damaged structures of blood platelets and vessels and affected their functions [35,36]. Acceleration of platelet aggregation of newborn calves with iron deficiency pointed at the increase of their receptors’ sensibility to outside stimulating impacts. Besides, active development of platelet aggregation in response to ristomicin was to be regarded as consequence of their sensibility increase to von Willebrand Factor. Besides, acceleration of platelet aggregation, developing in these animals indirectly, told about the increase of exchange processes of arachidonic acid with surplus thromboxan A2 formation [37] in their blood.

Weakening of vascular hemostasis functional abilities of animals with iron deficiency became apparent at lowering of vessels’ disaggregative features. It was evidently caused by the decrease of generation of prostacyclin and nitric oxide molecules [38] in their walls. At the same time, the examined calves had weakening of vessels’ anticoagulant and fibrinolytic abilities because of production depression of anticoagulant – antithrombin III and tissue activators of plasminogen in them.

Found acceleration of prothrombin period of newborn calves with iron deficiency pointed at the evident activity intensity of outer mechanism of plasma hemostasis starting. Its basis was activity increase of coagulation factors participating in it [39].Early APTP appearance was connected with activation of coagulation factors participating in the inner way of hemocoagulation. Acceleration of...
blood coagulation final stage pointed at the intensive change of fibrinogen into fibrin in examined calves [17].

Application of ferroglukin, fosprenil and hamavit combination made newborn calves with iron deficiency feel saturated with iron. It also brought positive dynamics of red blood and common animals’ state indices. The impact on examined calves’ bodies was accompanied by lowering of their LPO processes intensity in plasma what weakened its damaging influence on endothelium and liver platelets. After the application of ferroglukin, fosprenil and hamavit combination platelet aggregation of calves with iron deficiency weakened. It was mostly the consequence of positive impact of these means’ combination on innerplatelet LPO, receptor and postreceptor platelets’ functioning mechanisms [38]. Developing in these conditions increase of the period of platelet aggregation as a response to ristomicin, pointed at lowering of adhesion cofactor – von Willebrand Factor [19] in these calves’ blood.

In the result of applied impact animals, having at the beginning iron deficiency, got some strengthening of disaggregation, anticoagulant and fibrinolytic vessels’ features. On the basis of found changes, though, the control level of production intensity of prostacyclin, nitric oxide, antithrombin III and tissue activator plasminogen [29] in vascular endothelium of these calves wasn’t reached.

Found in examined animals on the background of applied impact slowdown of prothrombin period reflected normalization of hemocoagulation processes along the outer way mainly on behalf of production lowering of factors participating in it in liver [1]. Happened on the background of correction slowdown of initially accelerated APTT was accompanied by weakening of generation activity, normalization of coagulation factors and especially factor XII. Happened duration slowdown of hemocoagulation final stage, what was judged by thrombin period, pointed at weakening of fibrinogen transformation into fibrin till control level in examined calves.

As the result of our investigation it became clear that in the case of application of ferroglukin, fosprenil and hamavit combination to newborn calves with iron deficiency, we could provide normalization of hemocoagulation and positive dynamics of the rest hemostasis system components.

3. CONCLUSION
Iron deficiency of newborn calves can be considered as the model of hemostasis abnormality. With its help we can try different means and their combinations to cure hemostasiopathy. It is caused by the facts that newborn calves with iron deficiency are characterized by lowering of blood plasma antioxidant protection, intensity of LPO processes in it, increase of platelet hemostatic activity and hemocoagulation. It is also accompanied by depression of vascular wall abilities to inhibition of these processes. Because of the presence of iron deficiency in newborn calves it was justified to use iron preparation. For strengthening of its impact on a body there were prescribed stimulating metabolism and anabolism means – fosprenil and hamavit. In our study we found that in case of their application to newborn calves it’s possible to strengthen antioxidant protection, weaken LPO activity, decrease platelet activity, produce positive dynamics of hemostatic features of vascular wall and normalize plasma hemostasis. The received results allowed us to consider the usage of combination of fosprenil and hamavit on the background of ferroglukin to be sufficient for reducing of hemostasiopathy in newborn calves with iron deficiency.

REFERENCES


Table. Parameters of hemostasis in newborn calves with iron deficiency treated with ferroglukin, fosprenil and hamavit

<table>
<thead>
<tr>
<th>Consider indicators</th>
<th>Calves with iron deficiency, n=34, M±m</th>
<th>Control, n=29, M±m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>outcome after the correction</td>
<td></td>
</tr>
<tr>
<td>platelet aggregation with ADP, s</td>
<td>25.0±0.10</td>
<td>29.6±0.05</td>
</tr>
<tr>
<td>platelet aggregation with collagen, s</td>
<td>19.8±0.15</td>
<td>24.9±0.04</td>
</tr>
<tr>
<td>platelet aggregation with thrombin, s</td>
<td>37.9±0.21</td>
<td>46.6±0.16</td>
</tr>
<tr>
<td>platelet aggregation with ristomicin, s</td>
<td>22.5±0.16</td>
<td>38.6±0.07</td>
</tr>
<tr>
<td>platelet aggregation with epinephrine, s</td>
<td>68.2±0.25</td>
<td>85.3±0.06</td>
</tr>
<tr>
<td>VWDAI with ADP</td>
<td>1.44±0.003</td>
<td>1.58±0.003</td>
</tr>
<tr>
<td>VWDAI with collagen</td>
<td>1.33±0.005</td>
<td>1.46±0.008</td>
</tr>
<tr>
<td>VWDAI with thrombin</td>
<td>1.38±0.007</td>
<td>1.47±0.004</td>
</tr>
<tr>
<td>VWDAI with ristomicin</td>
<td>1.40±0.004</td>
<td>1.43±0.009</td>
</tr>
<tr>
<td>VWDAI with epinephrine</td>
<td>1.42±0.006</td>
<td>1.49±0.003</td>
</tr>
<tr>
<td>index value of vascular wall anticoagulation activity</td>
<td>1.23±0.006</td>
<td>1.28±0.005</td>
</tr>
<tr>
<td>index value of vascular wall fibrinolytic activity</td>
<td>1.23±0.009</td>
<td>1.30±0.006</td>
</tr>
<tr>
<td>activated partial thromboplastin period, s</td>
<td>27.0±0.29</td>
<td>39.8±0.33</td>
</tr>
<tr>
<td>prothrombin period, s</td>
<td>12.2±0.25</td>
<td>17.4±0.30</td>
</tr>
<tr>
<td>thrombin period, s</td>
<td>15.8±0.19</td>
<td>17.3±0.15</td>
</tr>
</tbody>
</table>
Legend: $p$ - reliability of differences of indicators between the control and the initial state of the calves with iron deficiency, $p_1$ – reliability of dynamics of indicators in calves with iron deficiency on the background of correction.