Assessing the efficacy of administration of ferroglukin, fosprenil, and hamavit, in combination, for the correction of hemostasis problems in iron deficient newborn calves

ABSTRACT
Iron deficiency may be observed in some newborn calves. This deficit negatively influences their growth and development to some extent due to problems with hemostasis (hemostasiopathy). The search for treatments for correction of hemostasiopathy in iron deficient newborn calves both aids in better veterinary treatment options, and in scientific advancements in hematology. This study evaluated the use of ferroglukin, a traditional therapy, in combination with two metabolism stimulators (fosprenil and hamavit) in newborn calves, and the efficacy of that combination therapy on hemostasis system indices. This study found that iron deficient newborn calves also have decreased plasma antioxidant protectability, an intensification of lipid peroxidation processes, an increase of thrombocyte hemostatic activity in the blood coagulation system, along with a decrease of the ability of vascular walls to bind it. In our study, we found that administration of a combination of ferroglukin, fosprenil and hamavit in iron deficient newborn calves resulted in improved plasma antioxidant and lipid peroxidation activity. Additionally, normalization of thrombocyte activity and positive dynamics of hemostasis vascular and plasma components were observed. Iron deficiency in newborn calves can be used as an animal model for hemostasis abnormalities. Using this animal model, researchers can evaluate potential treatments for hemostasiopathy. Study results demonstrated that the combined administration of fosprenil, hamavit, and ferroglukin was unsuccessful in correcting hemostasiopathy in iron deficient newborn calves.

Key words: newborn calves; iron deficit; hemostasis system; ferroglukin; fosprenil; hamavit.

1. INTRODUCTION
Research on cattle physiology has been directed at acceleration/enhancement of muscle growth and increase in productivity (meat and milk) [1], and evaluating treatments for different abnormalities/pathological states [2]. The accumulation of knowledge about physiological processes in calves at the very beginning of their ontogenesis is important because health and proper physiologic functions lays the foundation for subsequent milk and meat productivity in cattle. [3] Published research describes the leading role of hemostasis during ontogenesis, as blood components and hemostatic mechanisms influence heavily individual animal development [4,5] with the help of hemocirculation processes. Changes in hemostasis processes affect the activity of hemocirculation in tissues and organs, and, thus influence the common state of a body [6]. Previous published studies have noted that homeostasis deviations, especially in case of a young individual, can quickly increase hemostasis component activity leading to microcirculation disturbance [7]. Most studies in this area of research were conducted in humans [8,9,10]. Building on those previous research studies, [11,12] the authors of this study developed a conception of the presence of age-specific dynamics of hemostasis components activity, the most vulnerable mechanisms in the processes, and the potential for different influencing variants on the body, aimed at optimizing those hemostatic processes. Because of the great social and economic implications of thrombosis development associated with cardiac pathology [13,14] many researchers attention are still devoted to haemostatic changes in individual patients [15,16,17]. Those researchers investigated different aspects of hemostasiopathy pathogenesis on cardiac diseases. Various research studies have raised the possibility of correction of hemostasiopathy conditions, not only with the help of medicines [18,19], but also with the help of traditionally applied treatments [20,21]. Those discoveries have great significance for biological research, and allows more effective therapeutic treatments.
Given the earlier findings, we have both scientific and practical interest in assessing the hemostasis state of newborn calves with a variety of clinical dysfunctions, and in evaluating approaches to correction of a body’s state which can positively influence and reverse the signs of hemostasiopathy. We selected iron deficiency as a model of hemostasis abnormality, as it is often found in newborn calves, to study effective ways of correction of hemostasis system components. The iron deficient newborn calf model seemed to be appropriate, as it involves a decrease of hemoglobin content in blood and lowering of iron-bearing enzymes which suppress protein synthesis and activity of cellular functions [22]. Given that connection, iron deficiency was determined to be a state accompanied by changing of whole body physiology. Iron deficiency also contributes to abnormalities in all components of the hemostasis system.
Research aimed at correction of hemostasiopathy in individuals with iron deficiency has great scientific and practical significance. The decrease of hemostasis disturbances can serve the basis for development of effective therapeutic modalities for hemostasiopathy reduction in newborn calves as well as treatment for related diseases. Assessing the impact of a combination of a therapeutic iron supplement [23], fosprenil [24], and hamavit [25] (the latter two having earlier demonstrated their high biological activity as far as separate hemostasis components are concerned) may yield insights in effective treatment of iron deficiency hemostasiopathies. Therefore the aim of this research is to assess correction of hemostasis system component in iron deficient newborn calves through use of a combination treatment of ferroglukin, fosprenil, and hamavit.

2. MATERIALS AND METHODS

2.1 Materials

The experimental treatment group in this study included 34 newborn calves with iron deficiency, identified by hematological evaluation as having signs of erythropoiesis, and a decrease of iron content in their bodies (serum iron 12.3±0.10 umol/l, siderocytes 1.6±0.05%, hemoglobin 95.0±0.29 g/l, erythrocytes 4.1±0.13x10^{12}/l). The control group included 29 healthy newborn calves.

All research activities involving the animals were conducted in full compliance with ethical norms and recommendations on humanization of work with laboratory animals containing “The European Convention for the Protection of Vertebrate Animals Used for Experiments or in Other Scientific Purposes” (Strasbourg, 1986).

2.2 Methods

The state of lipid peroxidation (LPO) in animal plasma was determined based on the quantity of thiobarbituric acid–active products found in it, using a commercially available test kit [“Agat-Med” (Russia)]. An assessment of acylhydroperoxides was performed to assess the antioxidant activity level of the liquid part of blood [26]. A determination of the number of thrombocytes in the calves blood was obtained using Gorjaev’s chamber. Thrombocyte aggregation was determined by a visual micromethod [10] using the following inductors: ADP (0.5x10^{-5} M), thrombin (0.125un/ml), collagen (0.1mg/ml), ristomycin (0.8 mg/ml), epinephrine (5x10^{-6} M). Those determinations were performed using plasma containing a standardized quantity of thrombocytes (200x10^{3} tr.).

Disaggregation capabilities in vascular walls were assessed with temporal venous occlusion and the visual micromethod of thrombocyte aggregation registration [10] with all the applied inducing factors. We calculated the value of vascular wall disaggregation activity index (VWDAI) by dividing thrombocytes aggregation period on the background of venous occlusion with the time of thrombocytes aggregation appearance without use of occlusion. The index value of vascular wall anticoagulation activity of experimentally treated calves was also calculated by dividing antithrombin III activity after venous occlusion by its value before occlusion [27]. Vascular control over fibrinolytic blood activity was determined by calculating the index value of vascular wall fibrinolytic activity by dividing the time of euglobulinlysis before occlusion on lysis time after occlusion [27].

Plasma hemostasis was evaluated according to duration of activated partial thromboplastin period (APPT), prothrombinic period and thrombinic period, using generally accepted test methods [27].

Treatment of iron deficient newborn calves involved (1) a single intramuscular injection of ferroglukin (at a dose of 15mg of iron per 1kg of body weight); (2) an intramuscular injection of fosprenil (0.1mg/kg) each morning for 6 days; and (3) an intramuscular injection of hamavit (0.1ml/kg) each morning for 6 days. Assessment of each animal’s physical state (for treatment and control animals) was made two times – at birth and on the 7th day of life. The data were processed using the Student’s criterion (t). Statistical processing of data was performed using commercially available software “Statistica for Windows v. 6.0”, “Microsoft Excel”. Differences in data were considered reliable in case p<0.05.

RESULTS AND DISCUSSION

The iron deficient newborn calves, upon initial examination, were found to exhibit weakness, limpness, an absence of interest in the surrounding environment, paleness of the nasal passages. Those animals were noted to have increased LPO activity in plasma (acylhydroperoxide 3.42±0.012 μmol/l, thiobarbituric acid– active products 5.19±0.019 umol/l at value depression of blood liquid part antioxidant activity 22.0±0.23%). The values of these indices in control animals were 1.45±0.010 μmol/l, 3.46±0.012 umol/l and 33.7±0.15%, correspondingly.

Thrombocyte parameters in newborn calves blood corresponded to referenced norms. Thrombocyte aggregation in iron deficient animals turned out to be reliably increased (table). The earliest
thrombocyte aggregation appeared in response to collagen (19.8±0.15s), somewhat later it was enhanced with ADP and with ristomicin, and still later in response to thrombin (37.9±0.21s). The longest time for thrombocyte aggregation in iron deficient newborn calves occurred with epinephrine treatment (68.2±0.25s).

Newborn calves with iron deficiency were found to have a VWDAI decrease in relation to all the applied inductors (table). The lowest VWDAI value was associated with collagen, a slightly higher VWDAI value was observed with thrombin, and a still higher VWDAI value was observed with ristomicin, ADP and epinephrine. Animals in the treatment group were noted to have a decrease of vascular wall anticoagulant capabilities as determined by the index value decrease of vascular wall anticoagulant activity. Fibrinolytic features in the vessels of treated animals were also diminished – the index of vascular wall fibrinolytic activity decreased by 13.0%.

Iron deficient newborn calves were also characterized by an increase in APTT (on average 42.3%) and prothrombin period (on average 42.6%), combined with an extended thrombin period (on average 8.7%).

Treatment group (iron deficient) calves had improvement of their clinical state and their activity, and an increase of their serum iron level to the control values (29.3±0.12 umol/l). Following treatment with ferroglukin, fosrenil, and hamavit, treatment group calves were found to have a demonstrable decrease of plasma acylhydroperoxides (2.16±0.010 D_{29}1 ml, p<0.01) and thiobarbituric acid-active products (4.12±0.014 umol/l, p<0.01), and an increase of antioxidant activity to 29.1±0.09% (p<0.01).

Correction of iron deficiency via the combination drug therapy was accompanied by invarianility of thrombocyte numbers in their blood, and some slowing of thrombocyte aggregation. Thrombocytes in treated animals demonstrated greater aggregation with induction in plasma samples by collagen, ADF and ristomicin, and less actively by induction with thrombin and adrenaline (table).

Calves in the treatment group were noted to have evident VWDAI increase in relation to all the applied inductors (table). The minimum value was VWDAI with thrombin. Other VWDAI values were a bit higher and were approaching the control values. Iron deficient newborn calves that received the standard iron preparation in combination with metabolically active compounds were noted to have an increase of vascular wall anticoagulant activity, and an increase of vascular wall fibrinolytic activity of 9.5%.

As a result of the experimental treatment, we reached APTT slowing of 42.6% with a simultaneous decrease of prothrombin period of 42.6%. This allowed the improvement of the treatment group animals. Additionally, the length of the thrombin period of these calves, defining the activity of fibrinogen transition into fibrin, increased by 8.9% and reached reference control levels.

The realization of genetically-defined growth and development in living organisms takes place during the constant influence of numerous factors of environment and internal environment [28] on an organism. Physiological peculiarities and their influence are mostly expressed by the optimum of living beings blood content [29] especially as far as hemostasis system components’ activity is concerned [30]. Any disturbances in an organism are accompanied by negative dynamics of hematological indices [31,32] including parameters of hemostasis system [33,34]. It becomes clear, that in hemostasiopathy development in iron deficient newborn calves, there is not only an iron deficit, but also depression of plasma antioxidant protection which, as previous works showed, causes LPO activation. An increase of peroxidation in the plasma damages structures of blood platelets and vessels and affects their functions [35,36]. The acceleration of thrombocyte aggregation in iron deficient newborn calves points at the increase of receptor sensitivity to stimulating influences from the outside. Additionally, active development of thrombocyte aggregation in response to ristomicin in iron deficient calves should be regarded in consequence of their sensitivity increase to von Willebrand Factor. Acceleration of thrombocyte aggregation appearing in these animals indirectly suggests an increase of exchange processes of arachidonic acid with surplus thromboxan A₂ formation [37] in their blood platelets.

Weakening of vascular hemostasis functional capabilities in iron deficient animals became apparent with the lowering of vessel disaggregation features. It was evidently caused by decreased production of prostacyclin and nitric oxide molecules in the vessel walls [38]. At the same time, treatment group calves were noted to have a decrease of anticoagulant and fibrinolytic capabilities in vessels, likely due to depression of production of anticoagulant – antithrombin III and tissue activators of plasminogen, in the vessels.

Observed acceleration of prothrombin period of iron deficient newborn calves pointed to an apparent intensification of activation of outer mechanism of plasma hemostasis initiation, and had as its basis an increase of coagulation factors participating in the process [39]. Early APTT appearance was connected with activation of coagulation factors participating in the inner way of hemocoagulation.
Acceleration of blood coagulation final stage indicated a significant change of fibrinogen into fibrin in the treated animals [17]. Administration of the combination of ferroglukin, fosprenil and hamavit in iron deficient newborn calves restored serum iron levels to normal ranges, restored red blood cell numbers to normal ranges, and improved the health and physical status of the animals. Improvements in treated animals was accompanied by decreasing the intensity of LPO processes in plasma, and decreased its damaging effects on vascular endothelium and liver thrombocytes. Decreases in thrombocyte aggregation in iron deficient calves following administration of ferroglukin, fosprenil and hamavit is most likely the consequence of positive impact of these means’ combination on inner-thrombocyte LPO receptor and postreceptor thrombocyte functioning mechanisms [38]. An increase in the time of thrombocytes aggregation in response to ristomicin, pointed to the lowering of adhesion cofactor (von Willebrand factor) [19] in the blood of the treated calves. Iron deficient animals, following treatment, demonstrated some strengthening of disaggregation, anticoagulant and fibrinolytic vessels features. Findings in experimentally treated animals did not reach control animal levels of prostacyclin, nitric oxide, antithrombin III, and tissue activator plasminogen [29] in vascular endothelium of the control calves. In the treated group, a slowing of prothrombin period reflected normalization of humocoagulation processes mainly by lowering of factors involved in it in liver. [1] The slowing of initially accelerated APTP indicated a weakening of generation activity, and normalization of coagulation factors, especially factor XII. Observed slowing in the humocoagulation final stage, as assessed by the thrombin period, pointed to a decrease of fibrinogen transformation into fibrin to control levels in experimentally treated calves. The results of this study indicate that application of a combination of ferroglukin, fosprenil and hamavit in iron deficient newborn calves yields normalization of humocoagulation and positive dynamics in other hemostasis system components.

3. CONCLUSION
Iron deficiency in newborn calves can be considered as a model of hemostasis abnormalities. This model allows assessment of different treatment regimes for correction of hemostasiopathy. Iron deficient newborn calves are characterized by a lowering of blood plasma antioxidant protection, an intensification of LPO processes, an increase of thrombocyte hemostatic activity and humocoagulation, and depression of vascular wall capabilities to slow those processes. Iron deficiency in calves necessitate the administration of iron supplements/therapies. This study combined standard iron supplementation with drugs to stimulate metabolism and anabolism – fosprenil and hamavit. It was hypothesized that the use of fosprenil and hamavit in newborn calves would strengthen antioxidant protection, weaken LPO activity, decrease thrombocyte activity, produce positive dynamics of hemostatic features of vascular wall, and normalize plasma hemostasis. The results of this study indicate that the combination of ferroglukin, fosprenil, and hamavit for the treatment of iron deficiency hemostasiopathy in newborn calves was successful in correcting the hemostatis problems in those animals.

REFERENCES


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<table>
<thead>
<tr>
<th>Consider indicators</th>
<th>Calves with iron deficiency, n=34, M±m</th>
<th>outcome after the correction</th>
<th>control, n=29, M±m</th>
</tr>
</thead>
<tbody>
<tr>
<td>platelet aggregation with ADP, s</td>
<td>25.0±0.10</td>
<td>29.6±0.05</td>
<td>40.2±0.08</td>
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<td>platelets’ aggregation with collagen, s</td>
<td>19.8±0.15</td>
<td>24.9±0.04</td>
<td>31.4±0.08</td>
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<td>platelets’ aggregation with thrombin, s</td>
<td>37.9±0.21</td>
<td>46.6±0.16</td>
<td>53.8±0.07</td>
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<tr>
<td>platelets’ aggregation with ristomicin, s</td>
<td>22.5±0.16</td>
<td>38.6±0.07</td>
<td>48.0±0.12</td>
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<tr>
<td>platelets’ aggregation with epinephrine, s</td>
<td>68.2±0.25</td>
<td>85.3±0.06</td>
<td>97.6±0.06</td>
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<tr>
<td>VWDAI with ADP</td>
<td>1.44±0.003</td>
<td>1.58±0.003</td>
<td>1.68±0.008</td>
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<td>VWDAI with collagen</td>
<td>1.33±0.005</td>
<td>1.46±0.008</td>
<td>1.58±0.003</td>
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<td>VWDAI with thrombin</td>
<td>1.38±0.007</td>
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<td>VWDAI with ristomicin</td>
<td>1.40±0.004</td>
<td>1.43±0.009</td>
<td>1.51±0.006</td>
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<td>VWDAI with epinephrine</td>
<td>1.42±0.006</td>
<td>1.49±0.003</td>
<td>1.64±0.004</td>
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<td>index value of vascular wall anticoagulation activity</td>
<td>1.23±0.006</td>
<td>1.28±0.005</td>
<td>1.31±0.004</td>
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<tr>
<td>index value of vascular wall fibrinolytic activity</td>
<td>1.23±0.009</td>
<td>1.30±0.006</td>
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<td>activated partial thromboplastinic period, s</td>
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<td>39.8±0.33</td>
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<td>prothrombinic period, s</td>
<td>12.2±0.25</td>
<td>17.4±0.30</td>
<td>17.4±0.22</td>
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<tr>
<td>Thrombinic period, s</td>
<td>Control</td>
<td>Initial State</td>
<td>Correction</td>
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<td></td>
<td>15.8±0.19</td>
<td>17.3±0.15</td>
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<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
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</table>

Legend: p - reliability of differences of indicators between the control and the initial state of the calves with iron deficiency, p< sub 1 – reliability of dynamics of indicators in calves with iron deficiency on the background of correction.