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# Physiological Peculiarities of Vessels' Disaggregating Control over New-Born Calves' Erythrocytes

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### Authors' contributions

*This work was carried out in cooperation between both authors. Author TIG has developed the study, carried out the statistical analysis of the material and literature searches. Author SYZ wrote the minutes and the first draft of the manuscript. Both authors together carried out a set of material and conducted the analysis of the study. Both authors prepared the final version of the manuscript, read it and approved it.*

### Article Information

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## ABSTRACT

**Introduction:** The process of microcirculation determines significantly the common physiological status of calves including the new-born phase. The success of microcirculation process in tissues mostly depends on aggregation evidence of the most numerous population of regular blood elements – erythrocytes and the level of vascular wall's disaggregating control over them.

The aim is to determine peculiarities of vascular control over erythrocytes' aggregation in new-born calves.

**Materials and Methods:** The study was conducted on 32 calves of black-multicolored breed. All the calves were healthy and were got from healthy cows after normally progressing pregnancy. All the animals were kept on the territory of Central Russia. They were examined on the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>.

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6<sup>th</sup>, 7<sup>th</sup>-8<sup>th</sup> and 9<sup>th</sup>-10<sup>th</sup> days of ontogenesis. Lipid peroxidation in plasma was estimated by standard methods according to the amount of thiobarbituric acid-active products and the level of acylhydrazines with the account of plasma antioxidant activity. Spontaneous aggregation of erythrocytes and its response to temporary venous occlusion were also estimated. A single-factor analysis of variance was used with application of Fisher's F-reliability criterion. Differences in data were considered to be statistically significant at  $p < 0.05$ .

**Results:** In the course of the new-born phase the calves were noted to have a weak trend to strengthening of initially low erythrocytes' aggregative activity (the number of erythrocytes' aggregates on the 1<sup>st</sup>-2<sup>nd</sup> day was equal to  $8.0 \pm 0.14$ , on the 9<sup>th</sup>-10<sup>th</sup> day –  $8.2 \pm 0.19$ ). The presence of inactive erythrocytes' aggregation in new-born calves was mostly provided by strong disaggregating vessels' impact on them (the index of vessels' control over the quantity of erythrocyte aggregates on the 1<sup>st</sup>-2<sup>nd</sup> day was equal to  $1.11 \pm 0.004$ , on the 9<sup>th</sup>-10<sup>th</sup> day –  $1.17 \pm 0.003$ ). Found level of accountable indices is mostly connected with low activity of examined animals' plasma peroxidation (acylhydroperoxides on the 1<sup>st</sup>-2<sup>nd</sup> day were equal to  $1.53 \pm 0.26$  D<sub>233</sub>/1 ml, on the 9<sup>th</sup>-10<sup>th</sup> day –  $1.42 \pm 0.31$  D<sub>233</sub>/1 ml), what damaged weakly the membranes of erythrocytes and endotheliocytes.

**Conclusion:** New-born calves are characterized by physiologically favorable for microcirculation balance of erythrocytes' aggregation and vascular wall's disaggregating control over it. Received values of hematological indices can be useful for further researches of new-born calves' physiology.

*Keywords: The newborn phase; calves; ontogenesis; vascular wall; aggregation; erythrocytes.*

## 1. INTRODUCTION

It is acknowledged at present that vascular disaggregating impact on regular blood elements determines significantly blood rheological properties [1,2]. It has special physiological significance for the processes of microcirculation and, consequently, for metabolism on the level of tissues [3,4]. It becomes clear that the balance between aggregation and disaggregation of regular blood elements has great biological significance on all the stages of a living being's individual development [5,6] for the processes of growth, maturation and genetic potential's maximal exposure [7,8]. The state of these processes is paid more and more attention nowadays at conducting of physiological researches on productive animals [9] including cattle [10,11].

Notwithstanding the great physiological signification of vascular disaggregating control over regular blood elements, its ontogenetic dynamics in cattle is still poorly studied. There are just few separate reports about the state of aggregation and disaggregation of separate regular blood elements in calves [12,13]. It becomes clear that the processes of vascular control over erythrocytes' spontaneous aggregation play special biological role [14]. However, age-specific features of these processes remains unclear [15,16]. There is no information about the evidence of vessels' disaggregating impact on the processes of

erythrocytes' aggregation in calves during the new-born phase. For closing of existing gap in physiological knowledge we put the following aim in our research – to determine peculiarities of vascular control over erythrocytes' aggregation in new-born calves.

## 2. MATERIALS AND METHODS

The study was conducted in strict accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (adopted in Strasbourg on March, 18<sup>th</sup>, 1986, and confirmed in Strasbourg on June, 15<sup>th</sup>, 2006) and approved by the local Ethics Committee of Kursk Institute of Social Education, a branch of the Russian State Social University (record № 12, dated December, 3<sup>rd</sup>, 2015) and the local Ethics Committee of the All-Russian Scientific Research Institute of Physiology, Biochemistry and Animals' Feeding (record № 11, December, 4<sup>th</sup>, 2015).

The study was conducted on 32 new-born calves of black-multicolored breed. All the calves were healthy and were got from healthy cows after normally progressing pregnancy. All the animals were kept on the territory of Central Russia. The calves were examined five times – on the 1<sup>st</sup>-2<sup>nd</sup>, 3<sup>rd</sup>-4<sup>th</sup>, 5<sup>th</sup>-6<sup>th</sup>, 7<sup>th</sup>-8<sup>th</sup> and 9<sup>th</sup>-10<sup>th</sup> days of their ontogenesis.

The intensity of lipids' peroxidation (LPO) in plasma was estimated according to the quantity of thiobarbituric acid-active products with the help of reagents' set "Agat-Med" (Russia) and according to the level of acylhydroperoxides [17] taking into consideration the state of plasma antioxidant activity [18].

Spontaneous erythrocytes' aggregation in intact plasma and in plasma which was received after temporal ischemia of vascular wall, was registered with the help of light microscope in Gorjaev's box by calculating the number of erythrocyte aggregates, quantity of aggregated erythrocytes and number of non-aggregated erythrocytes [19]. The level of vascular control over erythrocytes' aggregation was determined according to its weakening in plasma received after temporal venous occlusion. It was made by application of sphygmomanometer's cuff on an extremity of a calf for 3 minutes and pumping of pressure in it on 10 mm merc. col. higher than the level of systolic arterial pressure [20]. In the course of dividing of the value of erythrocytes' sum in aggregates' formulation on the value of the given sum in plasma after temporal venous occlusion we calculated the index value of vessels' control over the sum of erythrocytes in the formulation of an aggregate (ICVSEA). In the course of dividing of aggregates' number in intact plasma on their number in plasma after temporal venous occlusion we found the index value of vessels' control over the quantity of erythrocyte aggregates (ICVQEA). In the course of dividing of free erythrocytes' number in plasma after temporal venous occlusion on the number of freely lying erythrocytes in plasma which was received without it, we calculated the index value of vessels' control over the quantity of free erythrocytes (ICVQFE).

Statistical processing of the obtained data was carried out using Statistics for Windows software, version 6.0 (Microsoft Excel). A single-factor analysis of variance was used with application of Fisher's F-reliability criterion. Differences in data were considered to be statistically significant at  $p < 0.05$ .

### 3. RESULTS AND DISCUSSION

The examined animals were found to have low level of LPO indices in plasma. At the same time, given parameters experienced a downward trend during the first 10 days of life: the content of acylhydroperoxides in plasma decreased on 7.7%, thiobarbituric acid-active compounds

lowered on 7.4%. The animals had it in the result of inclination development to the growth of plasma antioxidant activity which was summarily equal to 6.3%.

During the first 10 days of life the calves were found to have an inclination to the growth of spontaneous erythrocytes' aggregation. It was pointed by little increase of their erythrocytes' sum in the composition of an aggregate (4.7%), quantity of these erythrocyte aggregates (2.5%) at little lowering (2.4%) of free erythrocytes' quantity (Table 1).

During the first 10 days of life at conducting of the test with temporary venous occlusion the examined calves were noted to have some lowering of summary erythrocytes' number in aggregates' composition on 3.1% and these aggregates' quantity – on 2.8% what was accompanied by little rise of free erythrocytes' quantity. Found changes provided detection of little value growth of ICVSEA (on 8.5%), ICVQEA (on 5.4%) and ICVQFE (on 4.2%) (Table 1).

It is known that blood largely determines the functional state of the body. It becomes clear that physiological connection of somatic and hematological parameters is very close [21,22]. The optimum of erythrocytes' rheological properties is largely dependent upon continuous vessels' disaggregating impact on them. In this regard, to study the aggregation-disaggregation interactions in cattle blood in early ontogenesis [9] is of great scientific and practical interest.

The conducted research was devoted to estimation of disaggregating control of vascular wall over erythrocytes in calves in the course of the initial stage of their ontogenesis – the new-born phase. The examined animals were found to have stable high antioxidant plasma activity which provided effective moderation of LPO processes in it. Found low intensity of freely-radical processes in plasma of new-born calves undoubtedly promoted the optimum support of endotheliocytes' functional activity [23,24] which determined high antiaggregatory capabilities of their vascular wall on the whole [25,26].

During the new-born phase the calves were noted to have a little trend to strengthening of erythrocytes' aggregative activity. May be, shaping of erythrocytes' capability to form fragile links between them and globular plasma proteins lies in the basis of these changes in calves during the new-born phase. These links can act

**Table 1. Biochemical and hematological parameters of newborn calves**

Registered parameters	Age of calves, n=32, M±m					Dynamics of indicators
	1-2 days	3-4 days	5-6 days	7-8 days	9-10 days	
acyl hydroperoxides, D <sub>233</sub> /1ml	1.53±0.26	1.50±0.19 F=2.202 (p≤0.143)	1.48±0.23 F=2.344 (p≤0.131)	1.45±0.20 F=3.546 (p≤0.064)	1.42±0.31 F=3.757 (p≤0.057)	7.7%
TBA-active products, umol/l	3.62±0.12	3.58±0.16 F=1.377 (p≤0.245)	3.54±0.19 F=2.026 (p≤0.159)	3.49±0.10 F=3.261 (p≤0.076)	3.37±0.24 F=3.603 (p≤0.062)	7.4%
AOA, %	31.8±0.42	32.6±0.39 F=1.866 (p≤0.176)	32.9±0.36 F=2.287 (p≤0.135)	33.4±0.46 F=2.776 (p≤0.101)	33.8±0.28 F=3.660 (p≤0.060)	6.3%
The sum of all the erythrocytes in an aggregate	38.5±0.24	39.2±0.31 F=1.634 (p≤0.206)	39.6±0.39 F=1.898 (p≤0.173)	39.9±0.27 F=2.196 (p≤0.143)	40.3±0.38 F=3.460 (p≤0.067)	4.7%
The quantity of aggregates	8.0±0.14	8.0±0.18	8.1±0.09 F=1.266 (p≤0.264)	8.1±0.15 F=1.266 (p≤0.264)	8.2±0.19 F=2.276 (p≤0.136)	2.5%
The quantity of free erythrocytes	253.1±1.34	251.0±1.63 F=1.955 (p≤0.167)	250.1±1.42 F=2.448 (p≤0.122)	248.9±2.08 F=3.463 (p≤0.067)	247.2±1.85 F=3.769 (p≤0.056)	2.4%
The sum of all the cells in the assembly against the background of the temporary venous occlusion	32.8±0.22	32.7±0.26 F=1.852 (p≤0.171)	32.5±0.36 F=2.154 (p≤0.130)	32.1±0.46 F=2.647 (p≤0.103)	31.8±0.53 F=3.590 (p≤0.067)	3.1%
The number of erythrocyte aggregates against the background of temporary venous occlusion	7.2±0.10	7.1±0.16 F=1.248 (p≤0.257)	7.1±0.08 F=1.248 (p≤0.257)	7.0±0.07 F=2.305 (p≤0.226)	7.0±0.13 F=2.305 (p≤0.226)	2.8%
The number of available red blood cells against the background of temporary venous occlusion	298.6±2.63	299.2±1.96 F=1.635 (p≤0.238)	300.4±2.42 F=1.999 (p≤0.120)	301.7±2.58 F=2.548 (p≤0.087)	303.6±2.24 F=3.214 (p≤0.068)	1.7%

Registered parameters	Age of calves, n=32, M±m					Dynamics of indicators
	1-2 days	3-4 days	5-6 days	7-8 days	9-10 days	
ICVSEA	1.17±0.007	1.19±0.006	1.21±0.004	1.24±0.009	1.27±0.007	8.5%
		F=1.189 (p≤0.279)	F=2.007 (p≤0.161)	F=2.536 (p≤0.116)	F=3.709 (p≤0.058)	
ICVQEA	1.11±0.004	1.12±0.006	1.14±0.008	1.16±0.005	1.17±0.003	5.4%
		F=1.594 (p≤0.211)	F=2.192 (p≤0.143)	F=3.061 (p≤0.085)	F=3.695 (p≤0.059)	
ICVQFE	1.18±0.012	1.19±0.009	1.20±0.010	1.21±0.006	1.23±0.009	4.2%
		F=1.923 (p≤0.170)	F=2.606 (p≤0.111)	F=3.065 (p≤0.084)	F=3.361 (p≤0.071)	

Note: F – the value of Fisher's test when the indicators are compared with their values at the age of 1-2 days throughout the entire observation, p – possibility of unmistakable prognosis

as “bridges” between erythrocytes in the course of their spontaneous aggregation [27,28]. Besides, at the beginning of calves’ ontogenesis the threshold of erythrocytes’ disaggregation start was, evidently, rather low and it compensated the processes of erythrocyte aggregation [29,30]. Its effective realization in new-born calves, apparently, takes place, first of all, in the result of vascular walls’ active antiaggregatory impacts. That’s why, we can consider that the optimum of erythrocytes’ aggregation in their blood is mostly provided by vascular wall’s strong disaggregating impacts [31,32] and optimal electronegativity of erythrocyte membranes’ surfaces on behalf of the expression of some adequate quantity of proteins with a negative charge on it [33,34]. Efficient control in blood plasma of new-born calves over the formation of free radicals lowers the level of oxidative damages of cellular membranes [35] and large protein plasma molecules [36,37] which can link erythrocytes with each other in the course of aggregate-formation [38]. In these conditions active synthesis of prostacyclin and nitric oxide [39,40] in vascular walls of new-born calves suppresses effectively the processes of erythrocytes’ aggregation in blood. Linking on the surface of blood cells with their own receptors, they maintain physiologically minimal activity level of phosphodiesterase and adenylate cyclase in their cytoplasm. It provides optimal content of cyclic adenosine monophosphate and calcium [41,42] in it what suppresses erythrocytes’ aggregation in new-born calves [43,44].

Found changes of erythrocytes’ aggregative properties and vascular control over it in calves should be considered physiologically optimal for the beginning of the new-born phase. Given changes are, evidently, directed at the maintenance of high metabolic level [45,46] which is necessary for the beginning of ontogenesis. These changes also promote adaptation of a body to changing conditions of the environment [47,48].

#### 4. CONCLUSION

New-born calves are characterized by physiologically favorable for microcirculation balance of erythrocytes’ aggregation and vascular wall’s disaggregating control over it. Received values of hematological indices can be useful for further researches of new-born calves’ physiology.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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