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Eritrocytic Parameters of the Blood of Calves with Different Birth Weights

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Abstract

In the conditions of a dairy farm with the help of the standard clinical, instrumental and laboratory methods the research on particularities of Eritrocytic parameters of the blood of calves (n=299) with different birth weights was carried out. It is shown that Holstein calves with normal intrauterine growth, born with body weight from 36.5 to 29 kg, have no reliable differences in red blood cell count. With a 1 kg reduction in body weight, there is a trend towards hyperchromia, but an increase in weight deficiency promotes of hypovolemia, hypochromia, and polycythaemia. In newborns with a body weight of 27.9--26.9 kg, polycythaemia is derived from hypovolemia, but, with a more pronounced weight deficiency, the role of erythrogenesis disorder in the pathogenesis of the syndrome increases. Hypochromia is a consequence of hematopoietic organs dysfunction and transmembrane loss of hemoglobin. Herein membrane destruction is caused by the increase in the content of toxical metabolites in the blood (sorptivity of red blood cells by 10--12 percent), and by a higher level of adrenaline (modification coefficient of membranes by adrenaline by 20--30 %). This indicates that the response of the fetus to the factors that inhibit its development is similar to a metabolic response against stress. At strong underweight body (b.w. less than 25 kg) exhausting of adaptive capability is observed with the increase in the blood level of toxical metabolites (sorptivity of red blood cells by 15 %), level of membrane destruction (level of ectoglobular hemoglobin in 2,8 times), and heterogeneity of red blood cells. Thus, in newborns with a body weight of less than 29 kg, the significant disturbances in the structure and functions of red blood cells were revealed, which gave grounds for stating that they had antenatal hypotrophy. Herewith, the severity of hematological changes depends on the degree of weight deficiency. Initially, it is hypovolemia and the resulting polycythemia, but, erythropoiesis disorders, and destruction of their membranes with increased polycythemia, and the development of hypochromia occur with the increasing severity of hypotrophy.

Keywords: bovine, newborn calves, hypotrophy, red blood cells, polycythemia, hypochromia, hypovolemia.

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1. Introduction

The main mechanism of organism development in ontogenesis is the formation of its morpho- and functionally specific systems capable of supporting life activity, including resistance to various environmental influences. Blood plays a special role in this process, and its integration function forms the physiological unity of the organism. Hematopoiesis, consisting of many stages of cell differentiation, which result in the release of formed elements into the blood channels, begin in the middle of the first month of embryogenesis. By the moment of birth, the main hematopoiesis processes occur in the bone marrow, although some foci remain in the liver [1, 2]. It is at the hepatic stage of intrauterine growth of hemopoiesis that the megaloblastic type changes to the normoblastic type and becomes only extravascular, while red blood cells lose their nucleus, and the concentration of hemoglobin increases in them [3, 4]. Therefore, red blood cell count reflects the degree of morphofunctional maturity of the newborn-fetus.

Red blood cells are the main element of the blood gas transport system; they also perform the transport function by transporting proteins, amino acids, carbohydrates, hormones, enzymes, and trace elements, and take part in the processes of homeostasis and immune response [3, 4]. Under the influence on the body of adverse factors of the external and internal environment, red blood cells are the first to react by changing their structures and functions [5, 6], which may have a negative impact on homeostasis parameters, and a pathological process may be initiated [7]. Therefore, the information on the condition of red blood cells is important both for the body health assessment and from the point of view of identifying the threat of progression of pathology. These researches are most actual ones for newborns when there is a combination of risks of prenatal disorder of organogenesis, inappropriate existing conditions and failure of postnatal adaptive process and formation of functions of body systems. Therefore, the aim of our research was to study the features of erythrocytic parameters of the blood of newborn calves with different body weight to clarify the weight categories of their morpho-functional maturity assessment.

2. Materials and Methods

2.1. Animals

This study was approved by the Ethics Committee of FSBI, All-Russian Veterinary Research Institute of Pathology, Pharmacology, and Therapy and, during its implementation, the rules of humane treatment of animals were observed. In the conditions of the

industrial complex on milk production, where animals of Holstein breed were kept, we carried out the experiment, the object of which was the newborns (n=177), yielded as a result of non obstructed labor, having no signs of ugliness and intranatal asphyxiation. The mother was licking the calf during the first 5 minutes after birth, and then we placed the calf in a thermocase (38.5--0.0 ^{0}C) for artificial drying. After 65--75 and 420--430 minutes after birth, the calves were fed with 2 liters of colostrum from a suckling bottle. Newborns were kept in individual cages of preventive, where the relative humidity was in the range from 60 to 64 %, and the air temperature was from 18 to 25 °C.

2.2. Clinical examination

Pregnancy and fetal well-being were monitored using a portable ultrasound scanner (Draminski, Poland). The examination of the state of calves was carried out using common clinical and instrumental methods. Body weight of newborns was determined with the scales Momert 6681 (Hungary). According to the results of weighing, 6 experimental groups of 27--35 calves with different body weights were formed: 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 60 minutes after birth, from a jugular vein into IMPROVACUTER vacutainer tube with anticoagulant (K_3EDTA) to preserve its intact state (Guangzhou Improve Medical Instruments CO., LTD, China). Blood morphological parameters were studied with Micros 60 CT/OT (France) hematologic counter. The sorptivity of red blood cells was studied on A.A. Togaibaev et al. procedure [8], and the ectoglobular hemoglobin -- on O.A. Tonkoshkurova, etc. [9]. The state of membrane-receptor complexes on the surface of red blood cell membranes was evaluated by the method based on the comparison of the hemolysis degree of red blood cells in intact testings and, after modifiers putting in (adrenaline, and adrenoreceptor blocking agent), with the subsequent calculation of the modification coefficient (MCA) [10].

2.4. Statistical analysis

Math-and-stats of obtained data were made with SPSS version 22 (IBM Corp, Version 22.0, Armonk, NY, USA, 2013). The mean (M) and standard deviation (SD), statistical

significance (p), according to the Student's criterion, variation coefficient (C), correlation coefficient and determination coefficient were calculated.

3. Results and Discussion

The data in Table 1 show that, in comparison with the control (36.5--29.0 kg), in calves with a body weight of 28.9--28.0 kg there were no significant changes in the studied parameters, although there was the trend (p>0.05) to increase the level of hemoglobin (by 5.8 %, p>0.05), hematocrit (by 4.8 %), red blood cells (by 5.0 %), and MCHC (by 0.8 %).

Table 1: Blood parameters of newborns with a body weight between 27.0 and 36.0 kg (M±SD).

Parameters	Body weight, kg		
	36.5--29.0	28.9--28.0	27.9--27.0
Number, cattles	35	30	29
Red blood cells, 10^{12}/l	6.78±1.330	7.12±1.475	7.85±1.053 [2]
Ectoglobular hemoglobin, g/l	0.44±0.176	0.42±0.156	0.42±0.148
Hemoglobin, g/l	112.0±25.03	118.5±27.43	130.9±25.61[2]
Hematocrit, %	41.5±6.10	43.5±6.37	46.8±5.50[2]
MCH, pg	16.51±1.766	16.65±2.862	16.67±4.915
MCHC, %	26.98±4.475	27.19±1.131	27.95±8.100
MCV, μm [3]	61.2±9.666	61.09±6.062	59.6±11.42
MCA	1.23±0.049	1.25±0.039[1]	1.54±0.038[3]
Sorptivity of red blood cells, %	39.0±12.46	38.7±9.72	39.3±10.09

Note: Here and below, the difference with data the calves of first weight range (36.5--29.0 kg) is statistically significant: «1» -- p<0,05; «2» -- p<0.01; «3» -- p<0.001.

Polycythaemia was detected in animals with a body weight of 27.9-27.0 kg, which is indicated by the increase of hematocrit by 12.8 %, hemoglobin -- by 16.9 %, and red blood cells -- by 10.3 %, compared with control (36.5-29.0 kg). However, this syndrome should be considered as "false" in these calves, because it is caused by hypovolemia, but not by a change in erythropoiesis processes, which is confirmed by a high level of hematocrit against the background of preservation of mean corpuscular volume of red blood cells, the content and concentration of hemoglobin in them [11]. MCA values were increased by 25.2 %, which indicates a high level of endogen catecholamines in the blood (adrenaline, noradrenaline), which activate the adrenergic receptors on red blood cells membranes [12--14]. However, there was not yet the hyperadrenia systemic effect, in particular, changes of red blood cells membranes permeability. While newborns with

a body weight of 26.9-26 kg (Table 2) have not only an increase in MCA (by 30.1 %) but also ectoglobular hemoglobin (by 31.8 %), which is caused by changes in red blood cells membranes permeability in response to increased adrenaline concentration [15]. Hemoglobin content in hemolysate decreased by 17.9 %, as a result of the loss of intracellular hemoglobin.

Table 2: Blood parameters of newborns with a body weight between 24,0 and 26,9 kg (M±SD).

Parameters	Body weight, kg		
	26.9--26.0	25.9--25.0	24.9--24.0
Number, cattles	29	27	27
Red blood cells, 10^{12}/l	5.93±1.841[1]	5.58±1.097[2]	5.20±0.886[3]
Ectoglobular hemoglobin, g/l	0.58±0.225[1]	1.17±0.308[3]	1.25±0.217[3]
Hemoglobin, g/l	92.0±15.62[3]	84.0±14.25[3]	70.0±10.00[3]
Hematocrit, %	37.0±11.07	39.9±5.70	34.0±7.20[3]
MCH, pg	15.5±2.43[1]	15.0±4.06[1]	13.45±3.84[3]
MCHC, %	24.8±3.93[1]	21.0±5.63[3]	20.57±5.86[3]
MCV, μm [3]	62.4±9.36	71.5±19.30[1]	65.4±18.96
MCA	1.60±0.072[3]	1.31±0.03	0.85±0.039[3]
Sorptivity of red blood cells, %	40.9±6.50	44.5±7.54	45.1±9.82[1]

Note: Here and below, the difference with data the calves of first weight range (36.5--29.0 kg) is statistically significant: «1» -- p<0,05; «2» -- p<0.01; «3» -- p<0.001.

At the same time, animals had 12.5 % less red blood cells, 10.8 % less hematocrit, 6.1 % less MCH, and 8.1 % less MCHC, respectively, compared to controls. The mean corpuscular volume of red blood cells was not significantly changed, and normotocytes prevailed in the common cell pool. Therefore, there is no reason to state violations of erythropoiesis, the presence of which is not in doubt in animals with a body weight of 25.9--25.0 kg. The increase of 16.8 % in mean corpuscular volume of red blood cells, a decrease in MCH by 8.8 %, and MCHC by 22.2 % were indicated, the variability of which was significant (C = 27.0--28.3 %), in addition to the red blood cells membranes damaged structure and functions, which is confirmed by the increased values of MCA and sorptivity of red blood cells. At the same time, the percentage of normocytes was only 17 %, while the percentage of micro- and macrocytes was 25 and 58 %, respectively; i.e., it is obvious that red blood cells' heterogeneity has increased, which confirms the violation in erythropoiesis processes [16]. Newborns in this group had a 2.7-fold higher ectoglobular hemoglobin rate than in controls, which was one of the reasons for the low level of hemoglobin (by 25 %). Hypochromia was also facilitated by the impairment of hematopoietic organs function, as indicated by the above changes

in red blood cells indexes and a decrease in their number (by 17.7 %). Mean corpuscular volume of red blood cells, which was higher by 16.8 % than that of normotrophicsis, is focused on the compensation of this pathological phenomenon (hypochromia). More pronounced hematological abnormalities were found in calves with a birth weight of less than 24.9 kg, in which the level of red blood cells was decreased by 23.3 %, hemoglobin by 37.5 %, and hematocrit by 18.1 %. The deficit of hemoglobin content (18.5 %), and hemoglobin concentration (23.8 %) in red blood cells indicates of a pronounced hypochromia, but the tendency to decrease MCV (6.9 %, $p>0.05$) indicates of depletion of the compensatory potential of hematopoietic organs [6, 17]. The significant variability of the calculated indices (C=28.5-30 %), as well as the disturbed structure of the red blood cells pool, in which 17 % -- normocytes, 28 % -- microcytes, and 55 % -- macrocytes, indicate of anisocytosis. A low MCA value (by 30.9 %) suggests a decrease in the level of adrenaline in the blood, which is probably due to adrenal insufficiency [18, 19], and indicates a decrease in adaptive capacity, in particular, stress tolerance in newborns body [20]. In this case, despite the weakening of the role of adrenaline in red blood cells membranes modification, the degree of their destruction has increased, as indicated the ectoglobular hemoglobin high level (by 2.8 times).

When choosing the variant of the strength of relationship analysis between calves' birth weight and erythrocytic parameters of their blood, we took into account that we have the association of experimental material (the algorithm of antenatal development), and sampling with null-hypothesis, since all animals were born alive. It is obvious that the differences in studying parameters are a part of an integral host response reflecting the metabolic profile in newborns with different levels of morphofunctional maturity. Therefore, to evaluate their diagnostic yield, we used a linear regression model with the calculation of correlation coefficient and determination coefficient [21].

The results of the statistical analysis showed (Table 3) that there is a close correlation relationship between the basic parameter and the majority of the studied indicators, but a moderate correlation showed with MCA, and a significant correlation with the level of hematocrit and mean corpuscular volume of red blood cells. It turned out that the changes in newborn body weight have the most significant effect on sorptivity of red blood cells, on ectoglobular hemoglobin, and on MCHC, but have the average level of effect on the other parameters, except for MCA formation, where the basic parameter participation is minimal. The mathematical analysis took into account that there could not be the linear modulation in the pair "body weight -- hematological index" due to the adaptive-compensatory potential presence in the body, changing the pathophysiological effect of hypotrophy. Therefore, we assessed the merit of fit of factual

TABLE 3: Indicators of the linear regression between birth weight and erythrocytic parameters of calves.

Paired up "Body weight" indicator	Linear regression (forum of dependence)	Coefficient		Mean error of approximation, %
		correlation	determination	
Red blood cells	y=0.412x–4.523	0.764	0.584	6.431
Ectoglobular hemoglobin	y=–0.1846x+5.6045	-0.8850	0.7833	25.8046
Hemoglobin	y=10.0686x–165.5838	0.8179	0.6690	8.2109
Hematocrit	y=1.6600x–3.5400	0.6792	0.4613	6.5433
MCH	y=0.325x+7.353	0.832	0.693	2.197
MCHC	y=1.536x–15.955	0.887	0.787	4.478
MCV	y=–1.571x+105.176	-0.674	0.455	3.681
MCA	y=0.050x+–0.023	0.351	0.123	17.066
Sorptivity of red blood cells	y=–1.414x+78.729	-0.925	0.856	2.231

data to expect data, i.e. theoretical. For this purpose, the mean error of approximation was calculated, which showed that the closest value between the calculated and factual values, and thus have a relatively high prognostic perspective, was revealed from the side of the parameters (in descending order) of MCH, sorptivity of red blood cells, MCV and MCHC. In this respect, the parameters of red blood cells, hematocrit, and hemoglobin are also relevant. The level of other parameters studied depends on the weight of the newborns, but other factors are also involved in this formation. The weight of newborns also effects on the other studied parameters, but the other factors also participate in the process of parameters formation.

4. Conclusion

In cattle of Holstein breed, with a normal level of intrauterine growth, the minimum body weight at birth is 29 kg, and with its reduction, there are significant changes in erythrocytic parameters, what provide reasons for pronouncement of antenatal (congenital) hypotrophy. The severity of hematological failures depends on the degree of weight deficiency and the level of adaptive-compensatory potential. For example, there is a tendency to develop hyperchromia, when the body weight is reduced by 1 kg; but when the weight deficit increases; there are hypovolemia, hypochromia, and polycythemia. In animals with a body weight of 27.9--26.9 kg, syndrome of polycythemia

is due hypovolemia, but in a newborn with low weight, the role of defect of erythropoiesis increases in the pathogenesis of this syndrome. In addition to hematopoietic organs dysfunctions, transmembrane loss of hemoglobin plays an important role in the emergence of hypochromia. Herewith, membrane destruction is caused by the increase in the content of toxic metabolites in the blood and the higher level of adrenaline.

This indicates that the response of the fetus body to the factors, that slow its development, is similar to a metabolic response to stress. Therefore, at a high deficit of body weight (b.w. less than 25 kg), there is a cachexy of adaptive capacity with an increase of toxic metabolites level in the blood, the degree of membrane destruction, and heterogeneity of red blood cells. Thus, in newborns with body weight less than 29 kg, firm abnormalities of red blood cells structure and functions were revealed. Herewith, the severity of hematological changes depends on the degree of weight deficiency. Initially, it is hypovolemia, and associated polycythemia, but with the increasing severity of hypotrophy, erythropoiesis is damaged, and red blood cells membranes are destructed with polycythemia increase, and hypochromia progress.

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Conflict of Interest

The authors have no conflict of interest to declare.

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