SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 9-20

UDC: 619:636.2:618.3: 591.111
DOI: 10.48077/scihor9.2023.09

Cow haemostasis and resistance of calves under hypoxia conditions

Mariia Kambur*
Doctor of Veterinary Sciences, Professor
Sumy National Agrarian University
40000, 160 Herasyma Kondratieva Str., Sumy, Ukraine
https://orcid.org/0000-0002-4864-5292
Andrii Zamazii
Doctor of Veterinary Sciences, Professor
Poltava National Agrarian University
36003, 1/3 Skovorody Str, Poltava, Ukraine
https://orcid.org/0000-0003-3138-0424
Viktor Kolenchenko
Postgraduate Student
Sumy National Agrarian University
40000, 160 Herasyma Kondratieva Str., Sumy, Ukraine
https://orcid.org/0009-00085-3472-9259
Oleksandr Demydko
Postgraduate Student
Sumy National Agrarian University
40000, 160 Herasyma Kondratieva Str., Sumy
https://orcid.org/:0000-0002-6433-315X
Yevheniia Livoshchenko
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40000, 160 Herasyma Kondratieva Str., Sumy, Ukraine
https://orcid.org/0000-0001-5826-4824

Article's History:
Received: 10.05.2023
Revised: 20.08.2023
Accepted: 27.09.2023

Abstract. Intensification of the livestock industry is impossible without effective animal reproduction. The solution to this problem is possible only if a viable offspring is obtained, and their body resistance and safety are increased, which determines the research relevance. In this regard, determining the influence of the haemostasis system and blood properties of cows on the growth and development of the foetus, and subsequently on the resistance of newborn calves, depending on the condition at birth, determined the research aim. The conditions of foetal growth and development

Suggested Citation:
Kambur, M., Zamazii, A., Kolenchenko, V., Demydko, O., & Livoshchenko, Ye. (2023). Cow haemostasis and resistance of calves under hypoxia conditions. *Scientific Horizons*, 26(9), 9-20. doi: 10.48077/scihor9.2023.09.

*Corresponding author

were found to be related to the activity of haemostatic factors and blood properties of cows. Hemocoagulation factors were active in animals that gave birth to calves in a state of hypoxia. The prothrombin time was 1.63, 1.40, 1.23 and 1.40 times lower, and the prothrombin haemostasis index was 1.53, 1.52, 1.35 and 1.46 times lower, respectively than in cows that gave birth to functionally active calves ($p<0.01$). The thrombin time of haemostasis, partially activated thrombin time and fibrinogen content in cows of the experimental groups were higher than in animals of the control group. The blood viscosity of cows in the control group was lower ($p<0.01$), and the blood coagulation of cows in the experimental groups was faster ($p<0.05$). The increase in coagulation properties of the blood of cows that gave birth to calves in a state of hypoxia occurred against the background of a decrease in the activity of the fibrinolytic system ($p<0.05$) and retraction of the blood clot. All this reduced the growth and development of the embryo and foetus. The placental ligamentous connection of foetuses born with signs of hypoxia was significantly greater than that of functionally active calves. The intensity and size of embryo growth were higher in the group of functionally active calves ($p<0.05$). The activity of leukocyte defence factors, and, accordingly, the resistance of the organism of functionally active calves at birth was higher. The percentage of activated leukocytes in the blood of calves was practically the same, and the microbial count was higher in calves of the control group ($p<0.05$). The results obtained in the course of the research can be implemented in the process of obtaining and rearing calves, and offered to all livestock farms in Ukraine

Keywords: clotting; blood; leukocytes; indices; fibrin

INTRODUCTION

Carrying a foetus into the female body is accompanied by the formation of a complex biological system. It is believed that the maintenance of its gravity is ensured by the mother's body, the foetus, and the placenta. A disturbance in only one of these systems triggers compensatory reactions in the other to maintain the conditions for foetal development. In solving this problem, the haemostasis system is of great importance. The stability of haemodynamic processes in the mother-placenta-foetus system is a condition that ensures the normal course of pregnancy. Circulatory disorders in this system lead to the development of foetal hypoxia. Intrauterine hypoxia is manifested by fetoplacental insufficiency. It leads to impaired blood circulation in the uteroplacental basin and characterises the flow of blood to the foetus.

O.S. Yaremchuk and R.L. Varpikhovskyi (2021) believe that obtaining viable offspring and preserving the number of animals for reproduction is an urgent problem of livestock production. According to O.W. Stasyshyn (2020), the solution to the problem is possible only if a viable offspring with a high level of resistance is obtained. According to L.G. Tunikovska (2020), the environment continuously affects the mother-placenta-foetus system by various factors, causing a change in the relationship between the female and foetus. S. Kohli *et al.* (2021) point out that the mother's body creates conditions for the foetus. They depend on her ability to provide the foetus with components that affect the course and outcome of pregnancy. The development of the foetus in the mother's body contributes to increased blood clotting in the vessels and activation of haemostatic factors.

S. Vlasenko *et al.* (2021) established that during foetal gestation, the aggregation properties of red blood cells and platelets change in the female body, and the effectiveness of blood clot resorption decreases. The effect of hypoxia on the haemostatic system, foetal oxygen supply and the condition of newborn animals remains to be determined. The formation of the provisional organs in the body of females associated with foetal gestation is accompanied by physiological and morphological changes in all body systems. Studies have not determined the condition of newborn animals and the intensity of embryo growth. L.V. Koreyba (2019) proved that the intake of nutrients and oxygen to the foetus is an additional burden on the female body. This is evidenced by the haematological parameters of highly productive cows in the dynamics of the dry period. Insufficient supply of Oxygen to the foetus is manifested by hypoxia. It affects the activity of haemostasis factors and blood rheology. I.A. Zhabchenko *et al.* (2019), H. Hamada and S.G. Matthews (2019) considered the perinatal aspects of pregnancy maintenance in the context of chronic stress and proved that polycythaemia, increased platelet, and erythrocyte aggregation occur in the case of oxygen supply disorders. They also point out that a violation of oxygen homeostasis is accompanied by activation of the platelet link of the blood coagulation process, and the number of blood plates in the blood increases due to young platelet forms.

The haemostatic system responds to hypoxia by increasing the activity of coagulation processes and decreasing fibrinolytic activity. U. Ezechukwu *et al.* (2014) established a relationship between blood viscosity and platelet aggregation in the blood of pregnant women. Deviations from the physiological level of activity of haemostasis processes and indicators of blood reactivity in the female body during foetal gestation affect haemodynamic and haemostasiological processes. They disrupt processes adaptively related to the functions of the fetoplacental complex. The effect of hypoxia on the

utilisation of oxygen, the activity of coagulation and anti-coagulation factors, and blood properties have not been studied. According to G. Gonzales *et al.* (2017), an increase in blood viscosity and a decrease in the speed of its movement through blood vessels is manifested by stagnation in pregnant women. Tissues are saturated with fluid, and inflammatory processes occur. These processes are especially threatening for cows with multiple placentas. M.D. Kambur *et al.* (2018) believe that postpartum complications are accompanied by intense blood clot formation in the placental vessels, which is detected by analysing its appearance.

The impact of negative factors during critical periods of pre-and postnatal growth and development of the organism manifests itself at different times, especially during periods of dominant growth of organs of different systems (Livoshchenko, 2016). The mother's and foetus' organism forms an "adaptive response" to the action of negative factors, which is accompanied by a change in the functions of regulatory systems. The consequence of the "adaptive response" of the foetus may be a decrease in the reproductive capacity of its offspring, a violation of the metabolism of biologically active substances under the influence of androgens, which are found in significant quantities in the mother's body during prenatal stress It causes suppression of the body's immune response to an immunodeficiency state, shortens the gestation period, reduces foetal weight, and causes the development of pathological processes that complicate pregnancy and the birth of a foetus with a low level of body resistance (Matviichuk, 2022).

Such issues as the effect of hypoxia on the haemostasis of pregnant cows, foetal growth and development, and the resistance of newborn animals remained unaddressed by researchers, which in combination determines the subsequent focus of adequate measures to preserve newborn animals and increase their viability, which was the research aim.

MATERIALS AND METHODS

The experiments were carried out during 2021-2022 at the farm "Vitalia", Chernecha Sloboda village, Buryn district, Sumy region, private joint-stock company "RADHOSP "Shevchenkivskyi", Shevchenko village, Konotop district, Sumy region, and "Chernihiv Main Enterprise for Livestock Breeding" (Chernihiv, Chernihiv region). Blood samples were tested in the clinical diagnostic laboratory "Sekhmet", Sumy. The devices of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv, were used for the study. Indicators of oxygen homeostasis were determined in the clinical diagnostic laboratory of the Sumy Regional Children's Clinical Hospital (Sumy) and the laboratory of veterinary medicine (Lebedyn, Sumy region). Blood samples were prepared for the study at the Department of Anatomy, Normal and Pathological Physiology of Animals of Sumy National Agrarian University.

Cow parturition was observed during the study. According to the characteristics of the first breath, and its stability, the resulting calves and mother cows were divided into groups (n=5). Calves with an adequate respiratory process after birth were classified as functionally active animals. Cows – mothers of functionally active calves were assigned to the control group of animals (control). Calves born with meconium in the amniotic fluid, or a state of asphyxia and maternal cows were assigned to the first group (I). The second group (II) included mothers and their offspring who had inadequate, spontaneous respiratory movements after birth. The third group (III) included cows and calves born to them with spontaneous, adequate respiratory movements.

Blood samples were taken from the umbilical cord vessels in calves at birth and the jugular vein in cows. Asepsis and antisepsis rules were followed during the study. Blood samples were collected in vacuum containers with special tubes with heparin. Blood samples were urgently delivered on ice for testing in the laboratory. The diameter of the umbilical cord of newborn calves was measured, and body weight was determined by weighing. Growth, development of the embryo, foetus and resistance of the calves' organism were determined using the following formulas: placental connection was defined as the ratio of the umbilical cord cross-sectional surface to the body weight of the offspring; intensity of embryonic growth - by the value of the specific ratio of the body weight of newborns to the body weight of the parental pair; the value of embryonic growth, g, was defined as the ratio of the body weight of the offspring (kg) to the duration of gestation (days).

The oxygen balance of the animal organism was determined using a blood gas analyser Easy Blood JAS, Medica, (USA), platelet haemostasis – using a Coagulometer K 3002 OPTIC. Blood smears were prepared to determine the resistance of the calves' organism. A drop of blood was applied to the edge of a dry, degreased slide. The ground edge of the cover glass was brought to the drop at an angle of 45°. The angle formed by the glass was evenly filled with blood. By moving the right hand away from the body, the drop of blood was spread in a thin layer over the surface of the slide. The smear was air-dried and fixed. It was placed in a bath and methyl alcohol was applied to it with a pipette for 3-5 minutes. The smear was removed from the bath and dried. It was stained according to the Romanovski-Gimza method. The finished stain was pre-diluted with distilled water. For each ml of water, 2-3 drops of paint were added and poured onto the smear, which was kept in a humid chamber for 30-40 minutes. The stain was washed off with distilled water. The blood smear was air-dried. The leukocyte formula was counted in the prepared blood smears.

Indicators of leukocyte formula were used to determine the activity of leukocytes and indices of resistance of the calves' organism:

- Phagocytic activity – % of leukocytes involved in phagocytosis;
- The phagocytic count is the ratio of the number of bacteria absorbed to the number of cells involved in phagocytosis;
- Phagocytic index – % of phagocytes that absorbed bacteria;
- The phagocytosis completeness index was calculated by dividing the killed bacteria in the phagocytes by the total number of absorbed bacteria and multiplying by 100;
- Nuclear index was defined as the ratio of the percentage of monocytes and stick-nucleated neutrophils to segmented neutrophils;
- Resistance index = % lymphocytes: % segmented neutrophils;
- WBC, % – determined by dividing the number of active phagocytes by the number of leukocytes and multiplying by 100;
- CAF, 10^9/L – the number of active phagocytes as the product of the number of leukocytes divided by the number of lymphocytes and the phagocytic index divided by 100;
- Microbial count, 10^9/l, was defined as the product of CAF and phagocytic count;
- Leukocyte intoxication index (LII)=(2xP+C)/((Mo+L) xE+1);
- Leukocyte shift index, % (LSI)=(M+MM+PN):CH;
- Leukocyte index=(4xmol+3xun+2xPN)+1)+CHx (0+1);
- Neutrophil-lymphocyte ratio (NLC)=neutrophils: lymphocytes;
- Neutrophil shift index=M+U+PN:CH.

All experimental studies were carried out by modern methodological approaches and in compliance with relevant requirements and standards, in particular, they meet the requirements of DSTU ISO/IEC 17025:2005 (2006). The animals were kept, and all manipulations were performed following the provisions of the Procedure for conducting experiments and experiments on animals by scientific institutions (Law of Ukraine No. 249, 2012), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986). The data were analysed using Microsoft Excel software. The reliability of the results was determined by the Student's criterion. Quantitative values were expressed as the arithmetic mean and its deviation (M±m).

RESULTS AND DISCUSSION

Various environmental factors affect foetal growth conditions. They disrupt the formation of the body's defence mechanisms, which makes it necessary to determine the resistance of newborn animals and carry out adequate correction. The process of embryogenesis is negatively affected by the disturbance of oxygen homeostasis in the body. Under the influence of hypoxia, during the germinal, embryonic and foetal periods, the body's supply of oxygen decreases and manifests itself in calves after birth (Table 1). The partial pressure of oxygen in the blood of newborn animals decreased depending on the severity of hypoxia by 1.45, 1.34, ($p<0.01$), 1.08 times and an average of 1.19 times ($p<0.05$) compared to functionally active calves. In cows of the first and second groups, the PO_2 value was significantly lower than in control animals ($p<0.05$).

PCO_2 in the blood of calves in hypoxia (groups I-III) was 1.73, 1.62, and 1.44 times higher than that of control calves ($p<0.01$), and 1.17 times higher than that of cows on average ($p<0.05$). The acidotic shift in blood pH of calves and cows of the experimental groups was not significant. Blood saturation of calves in the control group was 11.52±0.82 ml/dl. The oxygen saturation of calves' blood in the state of hypoxia was 1.52, 1.23, 1.13 times and on average 1.27 times less than O_2ct in functionally active calves, and A-aDO_2 on average 1.23 times ($p<0.05$).

Table 1. *Oxygen homeostasis in the blood of cows and newborn calves (M±m, n=5)*

Indicators	Measurement unit	Animal group				Average, per calve/cows I-III groups
		Control	I	II	III	
pH	log (C)	7.386±0.006	7.108±0.004	7.261±0.006	7.258±0.0015	7.209±0.006
		7.374±0.006	7.119±0.007	7.203±0.009	7.268±0.012	7.217±0.009
H+ ion content	mEq/l	52.01±2.031	63.02±3.011	54.03±2.01	49.01±2.05	55.33±3.01
		50.02±2.012	54.00±3.012	52.04±1.86	50.05±1.37	52.07±1.33
PO_2	mmHg	28.63 ± 0.97	19.84 ± 0.42**	21.31±1.23**	26.50±1.62	22.55±1.31*
		29.06±1.02	24.92±1.36*	26.02±1.36*	27.081±1.23	26.007±1.19*
PCO_2	mmHg	45.60±1.36	78.82±2.14**	73.85±3.78**	65.44±3.26*	72.70±3.20**
		46.40±0.74	58.12±2.46*	53.34±2.02	51.62±1.06	54.36±1.74*
TCO_2, general	mmol/l	28.81±0.79	32.42±1.07	31.14±0.78	30.86±0.96	31.47±1.86
		28.67±0.53	29.26±1.58	28.69±1.13	27.61±1.21	28.52±1.03

Table 1, Continued

Indicators	Measurement unit	Animal group				Average, per calve/cows I-III groups
		Control	I	II	III	
$\%SO_2$	%	80.66±4.02	66.82±2.24	74.86±2.74	82.08±3.31	74.35±2.55
		84.42±3.14	79.32±2.48	79.68±3.14	83.12±3.65	80.43±3.71
O_2 ct	ml/dl	11.25±0.97	7.85±0.62**	9.63±0.94*	10.42±0.68*	9.31±0.53*
		12.24±0.86	8.84±0.62	10.12±1.08	11.63±1.17	10.19±0.87
$A–aDO_2$	mmHg	58.18±2.04	43.89±1.43	45.30±1.71	52.60±2.30	47.26±1.71*
		60.02±1.68	55.49±1.63	56.58±1.46	58.70±2.52	56.92±2.14

Note: **p<0.05; **p 0.01; ***p<0.001 compared to the control group. Numerator – performance of calves. Denominator – indicators of cows*
Source: *compiled by the authors*

Under the influence of hypoxia, embryo growth is inhibited (Table 2). The placental ligament in calves exposed to hypoxia in the prenatal period was 1.91, 1.59 and 1.41 times higher (p<0.01) than in functionally active calves. The intensity of embryonic growth reached 0.035±0.001 g in calves of the control group. It was 1.45, 1.35, 1.30 and on average 1.35 times higher than in calves in the hypoxia condition. The embryonic growth rate was 0.120±0.002 g in the control group. In the embryos of the first – third groups, this indicator was 1.21, 1.13, 1.10 and on average 1.14 times less (p<0.05).

Table 2. *Indicators of embryonic growth (M±m, n=5)*

Indicators	Calve group				
	Control	I	II	III	On average per 1-3rd calve groups
Placental connection, mm^2/kg	0.341±0.002	0.653±0.003**	0.542±0.008**	0.483±0.006**	0.559±0.004
Intensity of embryonic growth, g	0.035±0.001	0.024±0.002*	0.026±0.002*	0.027±0.003*	0.026±0.002*
Embryonic growth rate, g	0.120±0.002	0.099±0.003*	0.106±0.002*	0.109±0.003*	0.105±0.001*

Note: **p < 0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

The appearance of the placenta indicates a violation of the conditions of foetal development under the influence of hypoxia.

In functionally active calves, the placenta is shiny. The vessels are clear, and filled, and capillaries are defined (Fig. 1).

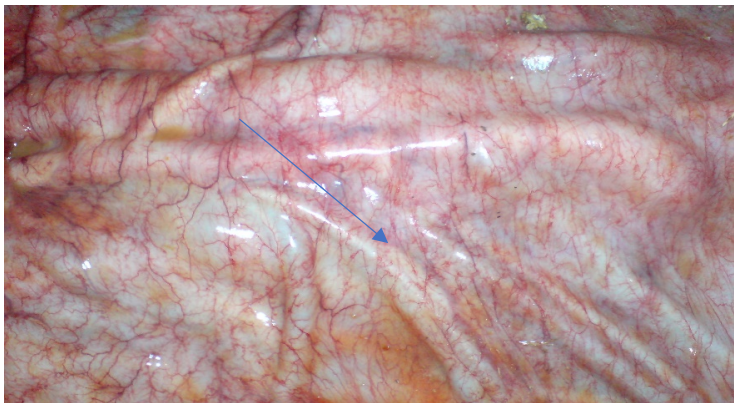

Figure 1. *Placenta of a functionally active calf*
Note: *placental vessels are clear, and capillaries are defined*
Source*: photographed by A.A. Zamaziy*

However, in calves born in a state of hypoxia, the placenta is not coloured, there are foci of haemorrhage and inflammation. The vessels are not clear, and the capillary system is not defined (Fig. 2).

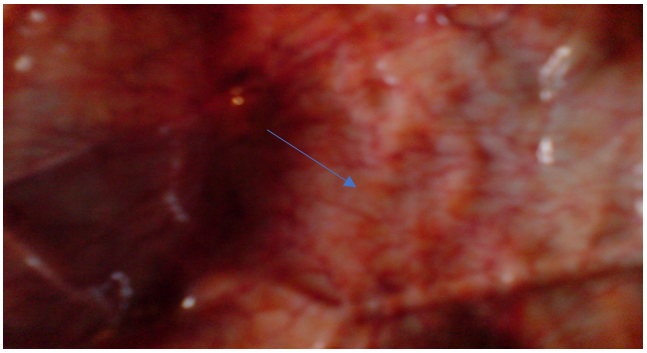

Figure 2. *Calf placenta in a state of hypoxia*
Note: *placental vessels are not clear, and the capillary system is vague*
Source*: photographed by A.A. Zamaziy*

The haemostasis of cows whose offspring were born with signs of impaired O_2 homeostasis was characterised by an increase in the activity of coagulation processes (Table 3). The prothrombin time was 48.20±1.42 s in cows of the control group. It was 1.63, 1.40, 1.23 and on average 1.40 times longer than in cows of the experimental groups (p<0.01). The prothrombin index of cows of groups I-III was 1.53, 1.52, 1.35 and on average 1.46 times lower than that of control cows (p<0.01). The thrombin time of haemostasis in control cows lasted longer, by 1.29, 1.23, 1.17 and 1.23 times on average (p<0.05). In cows of the control group, the partially activated thrombin time was 55.80±2.40 s. It was 1.34, 1.27, 1.40 and on average 1.28 times higher than the APTT of haemostasis in cows of the experimental groups.

Table 3. *Indicators of cow haemostasis (M±m, n=5)*

Indicators	Groups				
	Control	I	II	III	On average for animals of groups I-III
Prothrombin time, (TI), s	48.20±1.42	29.59±1.23**	34.24±1.81**	39.12±1.96*	34.32±1.21**
Prothrombin index, (PI), %	49.72±1.64	32.38±1.22**	32.89±1.53**	36.71±0.93**	33.99±0.83**
Internationally Normalised Ratio (INR), %	2.56±0.22	2.11±0.23	2.43±0.17	2.56±0.32	2.37±0.29
Thrombin time, s	36.84 ± 2.26	28.50±1.35*	30.09±2.17*	31.53±2.52*	30.04±1.94*
Activated partial thrombin time (APTT), s	55.80±2.40	41.60±1.90*	43.90±2.05*	45.10±1.82*	43.53±1.77*
Fibrinogen, g/l	2.46±0.18	3.93±0.29**	3.45±0.21*	3.14±0.28*	3.51±0.42*

Note: **p<0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

Fibrinogen was determined in the blood of cows of groups I-III – 3.93±0.29 g/l, 3.45±0.21 g/l and 3.14±0.28 g/l. Its content was at the level of 2.46±0.18 g/l in cows of the control group. It was 1.61 (p<0.01), 1.41 and 1.28 times (p<0.05) less than in animals of the experimental groups. A probable increase in the content of fibrinogen in the blood, and activation of the coagulation process, affected its properties (Table 4).

Table 4. *Properties of cow blood (M±m, n=5)*

Indicators	Animal group				On average per 1-3rd calve groups
	Control	I	II	III	
Blood viscosity, Pa*s	5.68±0.92	7.19±0.83*	6.79±0.37*	6.38±0.82	6.79±0.63*
Blood coagulation rate, s	402±8.00	361±6.00*	374±9.00*	386±5.00*	373.67±6.03*

Table 4, Continued

Indicators	Animal group				On average per 1-3rd calve groups
	Control	I	II	III	
Fibrinolysis, min	5.35±0.25	6.38±0.32*	6.21±0.43*	5.68±0.36	6.09±0.43*
Thrombotest, art	4.22±0.34	5.81±0.641*	5.29±0.51*	5.17±0.32*	5.42±0.48*
Retraction of a blood clot, %	64.18±2.24	53.84±3.2*	57.63±2.15	59.41±1.81	56.96±1.79*
Platelet adhesion, %	37.88±1.24	48.28±2.04*	46.65±2.38*	42.19±1.95*	45.71±2.03*

Note: **p<0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

In cows of the control group, blood viscosity was 5.68±0.92 Pa*s. In animals of the first group, it was 1.26 times higher, and in cows of the second and third groups, it was 1.19-1.12 times higher than this indicator of the blood of functionally active calves (p<0.05). Blood clotting in cows of the experimental groups was 1.20, 1.16 and 1.12 times faster (p<0.05) compared to the control. The breakdown of a blood clot took 1.21, 1.18 and 1.08 times longer in cows of the first to third groups. The blood thrombotest of cows of groups I-III was 1.36, 1.26, 1.20 and on average 1.28 times higher than in animals of the control group (4.22±0.34 st). Blood clot retraction was 64.18±2.24% in cows of functionally active calves, which was 10.40%, 6.60%, 4.80% and on average 7.27% higher than in animals of the experimental groups. The ability of blood platelets in the control group to adhere was 10.52%, 8.76%, 4.40% and 7.89% less than in cows with hypoxia. The phagocytic activity of neutrophils in the blood of functionally active calves reached 78.00±3.05 % (Table 5).

Table 5. *Blood leukocyte activity of newborn calves (M±m, n=5)*

Indicators	Animal group				On average for calves of groups I-III
	Control	I	II	III	
Phagocytic activity of neutrophils, %	78.02±3.05	68.04±3.52	72.02±2.68	74.00±3.22	71.35±2.19
Phagocyte count	7.12±0.48	5.22±0.64**	6.16±0.58*	6.58±0.56	5.99±0.83*
Phagocyte index	72.14±2.62	62.25±1.39	70.28±3.46	72.42±4.02	68.327 ± 3.46*
Phagocytosis completion index, %	73.91±4.03	56.10±2.70*	62.22±3.60*	68.50±3.30	62.27±3.13
Nuclear index	0.458±0.08	0.819±0.14**	0.739±0.22**	0.619±0.16*	0.725±0.19**
Resistance index	1.18±0.26	0.64±0.07**	0.81±0.21**	0.94±0.08*	0.80±0.079**
Percentage of active leukocytes (WBC), %	29.23±1.97	20.49±1.25**	22.43±1.37**	24.87±1.25*	22.58±1.14*
The number of active phagocytes (CAF), 10^9/l	2.38±0.42	2.51±0.37	2.76±0.34	2.88±0.32	2.72±0.31
Microbial count, 10^9/l	19.33±1.54	12.69±0.96**	14.19±0.82**	16.28±0.94*	14.39±0.99**

Note: **p<0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

In animals in groups I-III, FAN was 10%, 6.0%, 4.0% and on average 6.67% less. The ability of phagocytes to neutralise foreign proteins, i.e., FF, was 7.12±0.84 in calves of the control group. This indicator of leukocyte activity in the blood of calves in the state of hypoxia was 1.37, 1.17, 1.08 and on average 1.19 times (p<0.05) less. The completeness of phagocytosis in the blood phagocytes of calves of groups I-III was 17.81%, 11.71%, 5.41% and on average 11.64% less than in calves of the control group (73.91±4.03%). The nuclear index of blood cells of control calves reached 0.46±0.08. It was 1.78, 1.61, 1.35 and on average 1.57 times (p<0.01) less than in calves in the state of hypoxia. The index of resistance in calves in the state of hypoxia was 1.80, 1.43, 1.24 and on average 1.46 times less (p<0.01) than in calves of the control group. The percentage of activated leukocytes, i.e., the number of leukocytes that participate in phagocytosis in the blood of calves of the control group was 1.43, 1.30 (p<0.01), 1.18 and on average 1.29 times higher than in animals of the experimental groups (p<0.05).

The absolute number of leukocytes involved in phagocytosis (CAF) was not significantly higher in

calves of the first – third groups, which is associated with a higher level of leukocytes in the blood of these calves. However, the microbial count in calves of the control group was significantly higher than in animals of the first to third groups (1.52, 1.36 ($p<0.01$), 1.15 times ($p<0.05$) and an average of 1.32 times ($p<0.01$)). The leukocyte index of intoxication (Table 6) of newborn calves of the control group was 2.66±0.84, which was 4.43, 3.69, 2.46 and on average 3.33 times higher than that of calves of the experimental groups ($p<0.001$). The leukocyte shift index was 0.74±0.078, 0.85±0.057, 0.94±0.171 and 0.84±0.068 on average, which was 1.55, 1.35, 1.23 and 1.36 times less than in functionally active calves, respectively. The leukocyte index, on average, in calves in a state of hypoxia (83.15±3.26) was not significantly higher than in calves of the control group – 82.55±3.17.

Table 6. *Resistance indices of newborn calves (M±m, n=5)*

Indicators	Animal group				On average for calves of groups I-III
	Control	I	II	III	
Leukocyte intoxication index (LII)	2.66±0.84	0.61±0.05***	0.73±0.059***	1.07±0.083***	0.80±0.052***
Leukocyte shift index (LSI), %	1.16±0.32	0.74±0.078	0.85±0.057	0.94±0.171	0.84±0.068
Leukocyte index	82.55±3.17	83.48±4.61	83.019±2.23	82.94±2.83	83.15±3.26
Neutrophil-lymphocyte ratio (NLC)	1.241±0.22	0.768±0.14**	0.96±0.22*	1.06±0.16*	0.93±0.13*
Leukocyte shift index	0.53±0.11	1.03±0.019**	0.98±0.062**	0.779±0.126**	0.93±0.171**

Note: **p<0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

The ratio of neutrophils to lymphocytes (NLC) was 1.61, 1.32, 1.22 and on average 1.36 times higher in calves of the control group, and the neutrophil shift index was 1.94, 1.85, 1.47 ($p<0.01$) and on average 1.75 times higher in calves in the state of hypoxia. Effective management of this industry is possible only if viable offspring are obtained and their safety is improved. M. Sharan and S. Shalovylo (2018) point out that identifying the causes and mechanisms of prenatal losses, as well as their correction, is an urgent task for production. According to M.M. Zhelavskyi *et al.* (2021), a long period of foetal development in cows is accompanied by the negative impact of various stimuli that disrupt the relationship in the mother-embryo, mother-embryo, and mother-foetus system. The obtained results of the research allow us to assert that a violation of the foetal oxygen supply negatively affects the body's homeostasis and is manifested by a violation of the respiratory process in newborn calves.

The effect of hypoxia on the foetus in the prenatal period is manifested by impaired embryonic growth. Under the influence of hypoxia, the placental ligament in calves increased and was larger ($p<0.01$) than in functionally active calves. The intensity of embryonic growth was higher in functionally active calves. It was, on average, 1.35 times higher than in calves born in a state of hypoxia. The amount of embryonic growth in the embryos of the first to third groups was on average 1.14 times less ($p<0.05$). It is believed that only an external examination of the umbilical cord at birth allows us to assume that the conditions for foetal growth and development are impaired and should be used in production. K.G. Pringle *et al.* (2018) believe that the indicators of embryonic growth and development indicate the unevenness of this process, which is caused by a decrease in the supply of oxygen. L. Tremetsberger and C. Winckler (2015) point out that the inequality of embryonic development is manifested by the peculiarities of the morphological and functional connection between the foetus and the mother's body. S.T. Cohen *et al.* (2020) proved that embryogenesis in the cow foetus is accompanied by changes in the biochemical structure of the cell protoplasm and the intensity of all life processes. At each subsequent stage (embryonic → pre-foetal → foetal) of embryonic development, the organism does not remain morphologically, biochemically, and physiologically the same as it was in the previous period.

Researchers determine the percentage of losses in the process of foetal growth and development without determining the causes and their manifestation in newborn animals. According to P.T. Ruane *et al.* (2017), during the embryonic period, losses are up to 45%, and in the foetal period, this figure reaches 7-9%. At the stage of morula formation, embryonic mortality is 2-3%. In cows, the most critical period is 7-18 days of pregnancy, they say. The proportion of postplacental embryonic death is 5-8%. R.C. West *et al.* (2019) found that losses in the embryonic period are based on disorders of trophoblast differentiation. The developmental delay and death of blastocysts occurs on day 15-18 of gestation. Tissues of dead blastocysts remain in the uterus for up to 35-45 days. Such animals do not show signs of sexual desire

and do not inseminate for the next three months. The length of the service period increases, the number of calves produced per year decreases, and production indicators are negatively affected.

Y.Y. Shin *et al.* (2018) found that an increase in the placental mother-foetus connection was observed in the setting of foetal growth retardation. According to J. Liao *et al.* (2019), it is accompanied by compensatory processes, such as increased placental blood flow, vasodilation, and umbilical cord hypertrophy. It is believed that the thickening of the umbilical cord to the body weight of newborn animals indirectly characterises the level of metabolism and, accordingly, the body's resistance. The intensity of embryonic growth and its size were significantly lower in animals that developed under the influence of hypoxia. The course of cows' pregnancy, and, accordingly, the foetus, is influenced by feeding conditions and technological stresses. Up to 22% of newborns die and up to 40% of abortions occur when pregnant cows are not fed properly.

Despite the presence of numerous nutritional factors that negatively affect the bodies of cows and foetuses, L.H. Duntas (2020) points out that only vitamin and selenium deficiencies in the diet can cause embryonic death. J. Moake & M. Baylor (2021) believe that hypoxia is the most common factor affecting the growth and development of the embryo, foetus, and foetus. Calf hypoxia is accompanied by a significant decrease in partial pressure of oxygen in the blood, its saturation, on average, by 1.27 times ($p<0.01$). We believe that these results alone indicate a variety of measures aimed at restoring the viability of calves after birth, depending on the state of hypoxia. The partial pressure of CO_2 and the content of hydrogen ions in the blood significantly increased. Indicators of oxygen homeostasis in the body of animals allow us to make assumptions about the conditions of foetal development. These data are in line with the results of a study by J.J. Saugy *et al.* (2016). They indicate that hypoxia negatively affects homeostasis in humans. However, the authors do not determine the impact of hypoxia on the body depending on the severity of the process. We believe that such an approach subsequently reduces the effectiveness of measures aimed at preserving newborns and is not adequate for the state of the organism.

The biological system that ensures the prevention of blood loss and the preservation of the physiological norm of circulating blood parameters is defined as the haemostasis system. In an uncomplicated pregnancy, the female body undergoes several adaptive reactions aimed at ensuring an adequate course of the gestational period of foetal development. Changes in the haemostasis system can cause complications for both the female and the foetus. A significant restructuring of the vital activity of pregnant females is associated with changes in the blood system and haemostasis. According to C. Whyte *et al.* (2017), activated haemostatic factors have the properties of inflammatory mediators. Compensatory changes in the body of pregnant women bring haemostasis into a state of unstable tense equilibrium. F. Agoreyo *et al.* (2016) found that an increase in blood viscosity in pregnant females, in combination with haemo concentration and increased fibrinogen content, is manifested by microcirculatory disorders. Perhaps this leads to a decrease in uteroplacental blood flow and creates conditions for foetal hypotrophy, as evidenced by the appearance of the placenta of calves born in a state of hypoxia. The smaller the diameter of the vessels, the higher the viscosity of the blood flowing in them. Optimisation of the blood flow rate in the interventricular space is possible by reducing the number of platelet cells, especially red blood cells and platelets.

The process of physiological haemodilution is aimed at increasing the volume of circulating blood plasma. The process is most clearly manifested in the second trimester of pregnancy. During this period, the second wave of trophoblast invasion ends, and the further course of pregnancy is programmed Factors that negatively affect embryogenesis reduce the level of anabolic processes and suppress blood supply to the foetus. They lead to placental dysfunction (placental insufficiency), which is characterised by functional and morphological changes in the placenta. Such changes in the placental complex cause various pathologies in the foetus. Delayed intrauterine development and foetal hypoxia are detected. The rheological properties of the blood of cows that have given birth to calves in a state of hypoxia differ significantly from those of cows with functionally active calves. They are characterised by an increase in blood viscosity, coagulation, fibrinolysis, platelet aggregation properties and reduced blood clot retraction. The appearance of the placenta indicates a violation of O_2 homeostasis in the foetus. In calves born with signs of hypoxia, the placental capillaries are blurred and illegible. There are foci of inflammation and haemorrhage.

According to V.O. Melnyk and O.O. Kravchenko (2018), the most common complication is foetal retardation, which is detected in 40% of cases Increased blood viscosity in blood vessels, impaired microcirculatory blood flow is the basis for impaired oxygen homeostasis in the foetus. In such animals, hypoxia is manifested by impaired external respiration. A prerequisite for adequate external respiratory function is timely pneumatisation of the lungs and even distribution of inhaled air. Decreased pneumatisation of the lungs occurs against the background of inhibition of alveolar fluid resorption and intensity of surfactant synthesis. V.O. Velichko (2022), M.M. Zhelavskyi *et al.* (2022) indicate that impaired oxygen homeostasis, foetal growth retardation, determined by low birth weight, reduces the body's resistance. The processes of haematopoiesis and blood profile formation are disrupted, and the body's

resistance decreases, which necessitates determining the state of the newborn's body and carrying out appropriate correction.

CONCLUSIONS

Depending on the severity of the process, newborn hypoxia is accompanied by a decrease in partial pressure of oxygen, blood saturation and an increase in PCO_2 ($p<0.01$). The placental connection was significantly increased under the influence of hypoxia ($p<0.01$), and the intensity and size of embryonic growth were higher in functionally active newborn animals ($p<0.05$). Blood clotting and blood clot breakdown in cows with functionally active calves was significantly longer and viscosity was lower than in cows that gave birth to calves in a state of hypoxia ($p<0.05$). The phagocytic activity of neutrophils significantly affected the phagocytic number, phagocytic index, and completeness of phagocytosis ($p<0.05$), indicating a high level of resistance of functionally active calves.

The leukocyte index of intoxication and the leukocyte shift index of functionally active calves were significantly higher ($p<0.001$) than in animals in a state of hypoxia. The percentage of activated leukocytes, i.e., the number of leukocytes that participated in phagocytosis in calves of the control group was 1.43, 1.30 ($p<0.01$), 1.18 and on average 1.29 times higher than in animals of the experimental groups ($p<0.05$). The microbial count was 1.52, 1.36 ($p<0.01$), 1.15 ($p<0.01$) and on average 1.32 times higher in functionally active calves ($p<0.01$). In the future, research should identify adequate schemes and develop devices to correct the state of the body of newborn calves, their impact on the body's resistance, and increase the viability and safety of calves.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Agoreyo, F.O., Izevbizua, M.O., & Innih, S.O. (2016). Izevbizua1 and silvanus O. innih2 fibrinogen, relative blood viscosity and haematocrit in the three trimesters of pregnant albino wistar rats. *African Scientist*, 17(4), 1595-6881.

[2] Cohen, C.T., Turner, N.A., & Moake, J.L. (2020). Production and control of coagulation proteins for factor X activation in human endothelial cells and fibroblasts. *Scientific Report*, 1(10), article number 2005. doi: 10.1038/s41598-020-59058-4.

[3] Duntas, L.H. (2020). Selenium and at-risk pregnancy: Challenges and controversies. *Thyroid Research,* 13, article number 16. doi: 10.1186/s13044-020-00090-x.

[4] European convention for the protection of vertebrate animals used for experimental and other scientific purposes. (1986). Retrieved from https://rm.coe.int/168007a67b.

[5] Ezechukwu, U.S., Nwovu, A.I., Akingbade, O.A., & Nwanze, C. (2014). Blood viscosity among pregnant women attending antenatal clinics in a tertiary hospital in Abakaliki. *Researcher*, 6(8), 18-21.

[6] Gonzales, G.F., Tapia, V., Gasco, M., & Carrillo, C.E. (2017). Maternal hemoglobin concentration and adverse pregnancy outcomes at low and moderate altitudes in Peru. *The Journal of Maternal-Fetal & Neonatal Medicine,* 25(7), 1105-1110. doi: 10.3109/14767058.2011.623200.

[7] Hamada, H., & Matthews, S.G. (2019). Prenatal programming of stress responsiveness and behaviours: Progress and perspectives. *Journal Neuroendocrinologia*, 31(3), article number e12674. doi: 10.1111/jne.12674.

[8] ISO/IEC 17025:2005. (2006). Retrieved from http://online.budstandart.com/ua/catalog/doc-page.html?id_doc=50873.

[9] Kambur, M.D., Zamazii, A.A., Livoshchenko, E.M., Plyuta, L.V., Pikhtyryova, A.V., Ostapenko, S.V., & Natyagly, O.M. (2018). The effect of hypoxia on oxygen homeostasis and acid-base balance of the body of the fetus and newborn animals. *Animal Biology,* 18(4), 40-46.

[10] Kohli, S., Singh, K.K., Gupta, A., Markmeyer, P., Lochmann, F., Gupta, D., Rana, R., Elwakiel, A., Huebner, H., Ruebner, M., & Isermann, B. (2021). Placental thrombo inflammation impairs embryonic survival by reducing placental thrombomodulin expression. *Blood,* 137(7), 977-982. doi: 10.1182/blood.2020005225.

[11] Koreyba, L.V. (2019). Hematological indicators in high-yielding cows in the dynamics of the dry period. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences Scientific Messenger LNUVMB. Series: Veterinary Sciences*, 21(93), 37-40. doi: 10.32718/nvlvet9307.

[12] Law of Ukraine No. 249 "On the Procedure for Carrying out Experiments and Experiments on Animals by Scientific Institutions". (2012, March). Retrieved from https://zakon.rada.gov.ua/laws/show/z0416-12#Text.

[13] Liao, J., Li, Y., Wang, X., Zhang, B., Xia, W., Peng, Y., Zhang, W., Cao, Z., Zhang, Y., Liang, S., Hu, K., & Xu, S. (2019). Prenatal exposure to fine particulate matter, maternal hemoglobin concentration, and fetal growth during early pregnancy: Associations and mediation effects analysis. *Environmental Research,* 173, 366-372. doi: 10.1016/j.envres.2019.03.056.

[14] Livoshchenko, E.M. (2016). Hemoglobin content in erythrocytes and blood oxygen capacity of calves in critical periods of postnatal life, depending on body weight at birth. *Scientific-Technical Bulletin of the State Research Control Institute of Veterinary Medicines and Feed Additives and the Institute of Animal Biology*, 17(2), 38-43.
[15] Matviichuk, D.M. (2022). Hemostasis and blood properties of cows during the dry period. *Science and Education a New Dimension. Natural and Technical Sciences*, 34(268), 15-18. doi: 10.32782/SEND-NT2022-268X34-03.
[16] Melnyk, V.O., & Kravchenko, O.O. (2018). *Obstetrics, gynecology and biotechnology of animal reproduction: Lecture notes.* Mykolaiv: MNAU.
[17] Moake, J.L., & Baylor, M.D. (2021). *Overview of Hemostasis*. Retrieved from https://www.msdmanuals.com/professional/hematology-and-oncology/hemostasis/overview-of-hemostasis.
[18] Pringle, K.G., Kind, K.L., Sferruzzi-Perri, A.N., Thompson, J.G., & Roberts, C.T. (2018). Beyond oxygen: Complex regulation and activity of hypoxia inducible factors in pregnancy. *Human Reproduction Update,* 16(4), 415-431. doi: 10.1093/humupd/dmp046.
[19] Ruane, P.T., Berneau, S.C., Koeck, R., Watts, J., Kimber, S.J, Brison, D.R, Westwood, M., & Aplin, J.D. (2017). Apposition to endometrial epithelial cells activates mouse blastocysts for implantation. *Molecular Human Reprodactien*, 23(9), 617-627. doi: 10.1093/molehr/gax043.
[20] Saugy, J.J., Schmitt, L., & Hauser, A., Constantin, G., Cejuela, R., Faiss, R., Wehrlin, J.P., Rosset, J., Robinson, N., & Millet, G.P. (2016). Performance changes after live high-train low in normobaric, hypobaric hypoxia. *Frontiers in Physiology,* 19(7), article number 138. doi: 10.3389/fphys.2016.00138.
[21] Sharan, M., & Shalovylo, S. (2018). Subsidiary mehtods of pregnancy diagnosis in dairy cattle – altemative for the uture. *Scientific Messenger of Lviv National University of Veterinare Medicine and Biotechnologies*, 20(89), 108-113. doi: 10.32718/ nvlvet8920.
[22] Shin, Y.Y., Jeong, J.S., Park, M.N., Lee, J.E., An, S.M., Cho, W.S., Kim, S.C., An, B.S., & Lee, K.S. (2018). Regulation of steroid hormones in the placenta and serum of women with preeclampsia. *Molecular Medicine Reports,* 17(2), 2681-2688. doi: 10.3892/mmr.2017.8165.
[23] Stasyshyn, O.V. (2020). A modern view of the function of the hemostasis system. *Ukrainian Medical Journal*, 1(6), 14-16.
[24] Tremetsberger, L., & Winckler, C. (2015). Effectiveness of animal health and welfare planning in dairy herds a review. *Animal Welfare,* 24(l), 55-67. doi: 10.7120/09627286.24.1.055.
[25] Tunikovska, L.G. (2020). Peculiarities of the impact of various stress factors on the organism of farm animals. *Livestock Breeding, Fodder Production, Preservation and Processing*, 111, 225-230. doi: 10.32851/2226-0099.2020.111.31.
[26] Velichko, V.O (2022). Physiological and biochemical response of the body of calves to non-specific stimulators of resistance. *Scientific and Technical Bulletin of the State Research Control Institute of Veterinary Medicines and Feed Additives*, 23(1), 43-47. doi: 10.36359/scivp.2022-23-1.05.
[27] Vlasenko, S., Rublenko, M., & Yeroshenko, O. (2021). Dynamics of hemostasiological parameters in cows during pregnancy, after birth and in obstetric, gynecological and orthopedic pathology. *Veterinary Medicine,* 1, 7-17. doi: 10.33245/2310-4902-2021-165-1-7-17.
[28] West, R.C., Ming, H., Logsdon, D.M., Sun, J, Rajput, S.K., Kile, R.A., Schoolcraft, W.B., Roberts, R.M., Krisher, R.L., Jiang, Z., & Yuan, Y. (2019). Dynamics of trophoblast differentiation in peri-implantation-stage human embryos. *Proceedings of the National Academy of Sciences,* 116(45), 22635-22644. doi: 10.1073/pnas.1911362116.
[29] Whyte, C., Mitchell, J., & Mutch, N. (2017). Platelet-mediated modulation of fibrinolysis. *Seminars in Thrombosis and Hemostasis,* 2(43), 115-128. doi: 10.1055/s-0036-1597283.
[30] Yaremchuk, S., & Varpikhovskyi, R.L. (2021). *Hygienic evaluation of keeping dry goods cows.* Vinnytsia: VNAU Center.
[31] Zhabchenko, I.A., Tertychna-Telyuk, S.V., Korniets, N.G., & Kovalenko, T.M. (2019). Perinatal aspects of maintaining pregnancy against the backdrop of chronic stress. *Reproductive Endocrinology,* 45, 29-33. doi: 10.18370/2309-4117.2019.45.29-33.
[32] Zhelavskyi, M.M., Kernychnyi, S.P., & Dmytriv, O.Ya. (2021). Cell death and its significance in reproductive pathology. *Ukrainian Journal of Veterinary and Agricultural Sciences,* 4(2), 18-26. doi: 10.32718/ujvas4-2.04.
[33] Zhelavskyi, M.M., Kernychnyi, S.P., Dmytriv, O.Ya., & Betlinska, T. (2022). Cellular aging and immunity. *Ukrainian Journal of Veterinary and Agricultural Sciences,* 5(1), 8-16. doi: 10.32718/ujvas5-1.02.

Гемостаз корів та резистентність організму телят за умов гіпоксії

Марія Дмитрівна Камбур
Доктор ветеринарних наук, професор
Сумський національний аграрний університет
40000, вул. Герасима Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-4864-5292

Андрій Анатолійович Замазій
Доктор ветеринарних наук, професор
Полтавський державний аграрний університет
36003, вул. Сковороди, 1/3, м. Полтава, Україна
https://orcid.org/0000-0003-3138-0424

Віктор Анатолійович Коленченко
Аспірант
Сумський національний аграрний університет
40000, вул. Герасима Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0009-00085-3472-9259

Олександр Сергійович Демидко
Аспірант
Сумський національний аграрний університет
40000, вул. Герасима Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-6433-315X

Євгенія Михайлівна Лівощенко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40000, вул. Герасима Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0001-5826-4824

Анотація. Інтенсифікація галузі тваринництва неможлива без ефективного відтворення тварин. Вирішення цієї проблеми можливе лише за умови отримання життєздатного приплоду, підвищення резистентності та збереженості його організму, що визначає актуальність досліджень. У зв'язку з цим визначення впливу системи гемостазу та властивостей крові корів на ріст і розвиток плода, а в подальшому на резистентність новонароджених телят залежно від стану при народженні визначило мету досліджень. Встановлено, що умови росту і розвитку плода пов'язані з активністю факторів системи гемостазу та властивостями крові корів. Фактори гемокоагуляції були активні у тварин, які народжували телят у стані гіпоксії. Протромбіновий час був у 1,63, 1,40, 1,23 і 1,40 раза нижчим, а протромбіновий індекс гемостазу – у 1,53, 1,52, 1,35 і 1,46 раза відповідно, ніж у корів, які народили функціонально активних телят ($p<0,01$). Тромбіновий час гемостазу, частково активований тромбіновий час та вміст фібриногену у корів дослідних груп були вищими, ніж у тварин контрольної групи. В›язкість крові корів контрольної групи була нижчою ($p<0,01$), а згортання крові корів дослідних груп було швидшим ($p<0,05$). Підвищення коагуляційних властивостей крові корів, які народили телят у стані гіпоксії, відбувалося на тлі зниження активності фібринолітичної системи ($p<0,05$) і ретракції кров'яного згустку. Все це знижувало ріст і розвиток ембріона та плода. Плацентарно-зв'язковий зв'язок плодів, народжених з ознаками гіпоксії, був достовірно більшим, ніж у функціонально активних телят. Інтенсивність та розміри росту ембріонів були вищими у групі функціонально активних телят ($p<0,05$). Активність лейкоцитарних факторів захисту і, відповідно, резистентність організму функціонально активних телят при народженні була вищою. Відсоток активованих лейкоцитів у крові телят був практично однаковим, а мікробне число було вищим у телят контрольної групи ($p<0,05$). Результати, отримані в ході досліджень, можуть бути впроваджені в процес отримання та вирощування телят, а також запропоновані всім тваринницьким господарствам України

Ключові слова: зсідання; кров; лейкоцити; показники; фібрин