The Hemostatic System and Its Variability during Pregnancy of Holstein Breed Animals

T. V. YANICH[1], M. A. DERKHO[1], A. A. TEGZA[2]
[1]South Ural State Agrarian University,
Ministry of Agriculture of the Russian Federation
Troitsk, Chelyabinsk,
RUSSIAN FEDERATION

[2]Department of Veterinary Medicine,
Kostanay Regional University named after A. Baitursynov,
Kostanay,
KAZAKHSTAN

Abstract: − Changes in the hemostaticsystem during pregnancy in heifers and heifer calves were studied, reference intervals for the thrombocytogram and the blood coagulation system during "physiological pregnancy" were established, the dependence of blood parameters on progesterone concentration and pregnancy trimester was assessed. The work was performed on heifer calves(pregnant) and heifers of the Holstein breed, from whom blood was taken for research. It was found that during pregnancy, the number of platelets and platelet crit in the thrombocytogram decreases by 3.36 and 3.62 times ($P<0.05$), but the number of large platelets increases by 39.87% ($P<0.05$). The clotting tendency of blood is determined by the variability of parameters associated with the fibrinogenesis process: thrombin time decreases by 19.88% ($P<0.05$) and the concentration of fibrinogen increases by 38.98% ($P<0.05$). This is facilitated by a decrease in antithrombin III level by 37.04%. The data obtained during a physiological pregnancy can be used as normative when assessing the condition of animals, which will allow timely to detect various abnormalities in the hemostatic system and to carry out appropriate preventive measures.

Keywords: − Hemostasis, pregnancy, pregnancy trimester, progesterone.

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1 Introduction

One of the most important biological systems of the animal body is the hemostatic system, the functioning of which is aimed at preserving blood flow and the integrity of blood vessels, as well as ensuring the possibility of stopping various bleeding in case of their possible damage, [1], [2]. In addition, it is one of the body's homeostatic mechanisms that determines blood volume and pressure in blood vessels and allows maintaining normal tissue perfusion, [3], [4].

The hemostatic system functions are provided by a set of interactions between the cellular components of blood with plasma and extracellular components of the blood vessel, which determines their dependence in the animal body on various factors of endogenous and exogenous origin. Pregnancy is no exception, which orients shifts in the hemostatic system along the "hypercoagulable pathway" due to changes in the levels of coagulation factors and blood fibrinolysis, which reach a maximum during childbirth, [5], [6] and reduce hemostatic complications during gestational and birth processes, [7].

Shifts in the hemostasis system are determined by the period of pregnancy and the degree of placental circulation development, [6]. For example, during pregnancy, the concentration of blood coagulation factors VII, X, VIII, fibrinogen and von Willebrand factor increases systematically, reaching a maximum during childbirth, [8], [9], which protects the mother's body from bleeding. In particular, fibrinogen, as a plasma procoagulant, binding to active platelets, ensures the pregnancy progression, [10]. This is due to its ability to affect the reactivity of platelets, [11], and, as a consequence, their secretory ability, which ensures the formation and maintenance of the integrity and endothelial functions of blood vessels, [5]. During pregnancy, the concentration of fibronectin in the blood also changes, reflecting the state of the endothelium in the blood vessels, [12]. This is due to the fact that fibronectin, on the one hand, is a ligand of platelet glycoprotein receptors, on the other hand, a component of the extracellular matrix of cells, [13], and due to this activates blood plates and their aggregative properties, determining clotting tendency of blood, [14]. Therefore, as pregnancy progresses, the number of platelets in the blood decreases, corresponding to its trimesters, [15]. This is also the result of the manifestation of numerous physiological changes in the mother's body, including an increase in blood volume in the circulatory system, platelet consumption by placental tissue, increase in the rate of sequestration and cell circulation.

According to [16], changes in the hemostatic system, including platelet and plasma coagulation components, during pregnancy are associated with the concentration of progesterone in the mother's body. In turn, platelet factors also affect the synthesis of hormones by placenta, [17], determining its functional state and fetal development.

Most aspects reflecting changes in the hemostatic system in the mother's body during pregnancy have been widely studied in humane medicine. Nevertheless, in veterinary medicine there is fragmentary information in the context of individual agriculture [3], [18], [19], [20], and laboratory animals, [21].

The purpose of this study was determined by studying changes in the hemostatic system in the body of pregnant heifers and heifer calves, establishing physiological reference intervals for the levels of thrombocytogram and plasma coagulation hemostasis in accordance with the trimester of "physiological pregnancy", assessing the dependence of blood levels on progesterone concentration.

2 Materials and Methods

Ethical Statement. The research design was approved by the Bioethics Committee of the South Ural State Agrarian University and was carried out in accordance with the principles of humane treatment of animals.

Animals. The work was carried out in 2021-2022 on the basis of Belagash LLP (Republic of Kazakhstan), specializing in dairy farming and using the Holstein breed of cattle for these purposes. The object of the study was heifers of a random age, which, when they reached a live weight of 340-360 kg (age about 15 months), were artificially inseminated by retrocervical method, [3]. The pregnancy was confirmed by ultrasound diagnostics using a DRAMINSKI iScan scanner (Poland) on the 45th day after insemination. After that, they were transferred to the section of pregnant animals. The method of keeping heifers and heifer calves is a loose box. Feeding two times a day. Animal feeding diets comply with the VIZh norms, [22], they include feed of their own production, made in their own feed shop and enriched with mineral and vitamin supplements. Starting from the 24-week gestation period, the number of concentrated feeds in the diet of heifers is reduced, providing free access to high-quality hay.

Sample Collection and Analysis. To perform the work from heifers inseminated at the age of 15 months, after confirmation of pregnancy and transfer to the section of pregnant heifers, an experimental group (n=20) was formed on the principle of approximate analogues. The study material was blood taken at the end of the I, II and III trimesters of pregnancy (12, 24 and 36 weeks. pregnancy) in the morning before feeding by vacuum method. Before taking the blood of animals of the experimental group, they were subjected to a clinical examination. To collect blood, vacuum tubes (VACUETTE) were used, designed for the study of the hemostatic system (blue lid) and biochemical studies (red lid).

Within 24 hours after the blood samples were taken, they were delivered to IV Smolin Laboratory LLP (city of Qostanay), using a thermal container. The thrombocytogram (platelets (PLT, 10^9/L), thrombocrit (PCT, %), mean platelet volume (MPV, fl), platelet distribution width (PDW, %), platelet large cell ratio (P-LCR, %)) was determined by conductometry and flow cytofluorometry using a hematological analyzer "Sysmex, XS-500I" (Japan). The value of blood clotting parameters (activated partial thromboplastin time (APTT, s), international normalized ratio (INR, ME), Quick's value (%), Quick's prothrombin ratio, thrombin time (s), fibrinogen (mg/L), antithrombin III (mg/L)) was determined using an automated coagulometer "ACLTOP 500" (USA) and a biochemical analyzer TBA-120FR (Japan). The progesterone concentration was determined by the enzyme immunoassay using ready-made sets of Progesterone-ELISA reagents (Xema, Germany) in accordance with the instructions for their use on the Infinite F50 enzyme immunoassay analyzer (Austria) equipped with the Hydroflex platform (Austria).

Statistical Analysis. Statistical analysis was carried out using the Statistica 6.0 software. It provided for checking the normality of the distribution of values in the sample using the Shapiro-Wilk test, calculating the average value (X) and its standard error (Sx). Additionally, a three-factor analysis of variance was performed, in which the following factors were used: trimester of pregnancy, progesterone, hemostatic profile. The level of statistical significance is assumed to be $P<0.05$.

3 Results

As is known, during pregnancy, natural physiological changes occur in the mother's body, [20], [23], which also affect the state of the hemostatic system.

Thus, shifts in the thrombocytogram of animals are associated with both pregnancy and its trimester. First, at the end of the first pregnancy trimester, an increase in the level of thrombocytogram parameters was observed in the blood of pregnant heifers compared to non-pregnant ones by 10.13-31.82% (Table. 1), reflecting the role of platelet factors in the formation of fetal development conditions. Second, platelet parameters changed statistically significantly during pregnancy. Thus, in the dynamics from the first to the third pregnancy trimester, the number of platelets and their volume fraction in the blood (thrombocrit) decreased by 3.36 and 3.62 times ($P<0.05$). At the same time, the mean platelet volume, which characterizes the degree of maturity of blood plates in peripheral blood, did not depend on pregnancy duration. At the same time, the width of platelet volume distribution in the bloodstream decreased by 23.08% ($P<0.05$), although the platelet large cell ratio (P-LCR) increased by 39.87% ($P<0.05$).

On average, during the pregnancy development, the number of platelets was 293.53±13.34 10^9/l, plateletcrit – 0.16±0.02%, mean platelet volume – 7.84±0.12 fl, platelet distribution width – 8.08±0.11%, amount of megalo-platelets – 9.44± 0.13% (Table 1).

An important part of the hemostatic system are blood parameters characterizing its clotting and anticoagulation ability, changes of which are aimed at maintaining blood flow and balance in hemostatic mechanisms, [1], [2].

Table 1. Platelets and their indices in heifers (non-pregnant, pregnant) and heifer calves Holstein breed (n=20), X±Sx

Indicator	Period of pregnancy, trimester/week.			On average during pregnancy
	I/12	II/24	III/36	
Platelets, (PLT), 10^9/l	528.00± 4.10*¹	195.30± 31.81*²	157.30± 4.10*²	293.53± 13.34
Plateletcrit, (PCT), %	0.29± 0.02*¹	0.11± 0.01*²	0.08± 0.02*²	0.16± 0.02
Mean platelet volume, (MPV), fl	7.93± 0.17*¹	7.80± 0.10	7.80± 0.09	7.84± 0.12
Platelet distribution width, (PDW), %	9.40± 0.12*¹	7.63± 0.14*²	7.23± 0.09*²	8.08± 0.11
Platelet large cell ratio, (P-LCR), %	7.70± 0.14*¹	9.86± 0.16*²	10.77± 0.09*²	9.44± 0.13

Note: *¹ - $P<0.05$ in relation to 15-month-old heifers; *² - $P<0.05$ in relation to the first trimester of pregnancy

Table 2. Indicators of blood clotting ability in heifers (non-pregnant, pregnant) and heifer calves of the Holstein breed (n=20), X ±Sx

Indicator	Period of pregnancy, trimester/week.			On average during pregnancy
	I/12	II/24	III/36	
Quick's prothrombin ratio	2.83± 0.07*¹	1.95± 0.04*²	1.78± 0.05*²	2.18± 0.05
Quick's prothrombin activity, %	41.97± 0.67*¹	42.53± 1.05	45.03± 0.75*²	43.17± 0.82
INR, IU	2.95± 0.03*¹	2.02± 0.04*	1.83± 0.06*	2.27± 0.04
APTT, s	56.26± 0.61	55.03± 0.39	53.43± 0.45	54.90± 0.48
Thrombin time, s	21.13± 0.18*¹	18.93± 0.02	16.93± 0.10*²	18.99± 0.11
Fibrinogen, mg/l	1,540.00± 16.83*¹	1,876.00± 49.96*²	2,140.24± 37.51*²	1,852.08± 34.77
Antithrombin III, mg/l	0.27± 0.02*¹	0.20± 0.03*²	0.17± 0.02*²	0.21± 0.02

Note: *¹ - $P<0.05$ in relation to 15-month-old heifers; *² - $P<0.05$ in relation to the first trimester of pregnancy

The main changes in the blood clotting system of heifers/heifer calves during pregnancy were determined not so much by changes in the magnitude of the coagulation process activation, as by an increase in the rate of its final stage (fibrin formation), as evidenced by a decrease in thrombin time by 19.88% (P<0.05) and an increase in fibrinogen concentration by 38.98% (P<0.05). This was also facilitated by a decrease in the level of anticoagulants (antithrombin III) by 37.04% (Table 2).

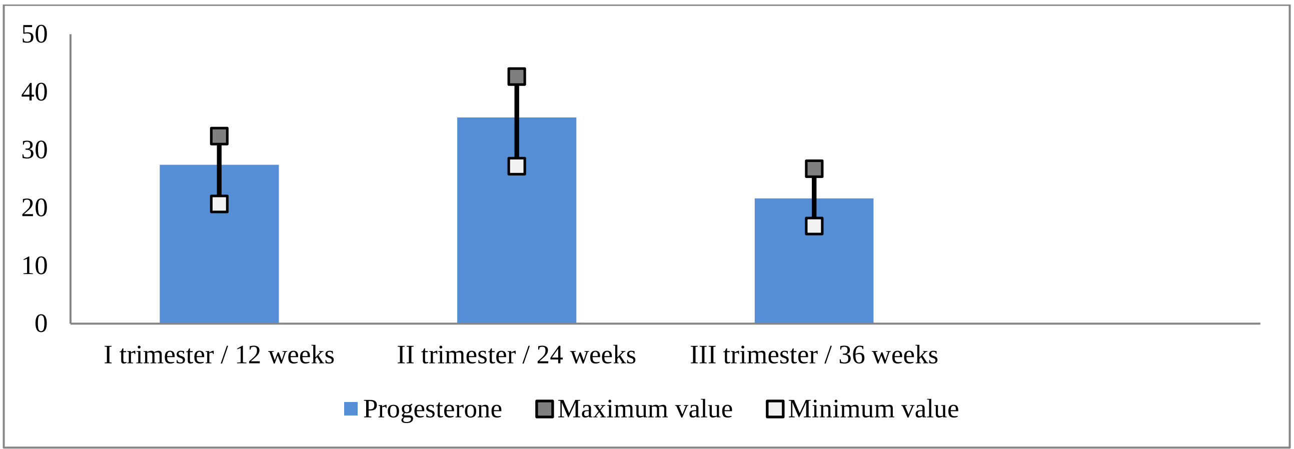

Fig. 1: Progesterone (nmol/l) in the blood of heifers (non-pregnant, pregnant) and heifer calves

On average, during the pregnancy period, the Quick's prothrombin ratio was 2.18±0.05, the Quick's prothrombin activity was 43.17±0.82%, the internationally normalized ratio (INR) was 2.27±0.04 IU, APTT – 54.90±0.48 s, thrombin time – 18.99±0.11 s, fibrinogen – 1852.08±34.77 mg/l, and antithrombin III – 0.21±0.02 mg/l (Table 2).

Table 3. Results of the dispersion analysis of the hemostatic system in heifers and heifer calves

Source of variation	Total sum of squares SS	Degree of freedom df	Standard deviation MS	F - criterion	F - critical	*P*
Main factors						
Pregnancy trimester	2,412.26	3	44.96	136.63	2.56	<0.05
Blood progesterone	14,470.71	3	36.01	140.57	2.56	<0.05
Hemostatic indicators	18,054.86	3	96,276.00	8,000.04	2.56	<0.05
Two-factor interactions						
Pregnancy trimester x progesterone	22,226.60	21	4,544.19	14.57	1.85	<0.05
Pregnancy trimester x hemostatic indicators	90,608.34	21	8,346.93	79.64	1.85	<0.05
Progesterone x hemostatic indicators	85,595.00	21	6,603.91	55.85	1.85	<0.05
Three-factor interaction						
Pregnancy trimester x progesterone x hemostatic indicators	355,950.90	539	6,789.91	46.74	1.40	<0.05

Progesterone plays an important role in pregnancy development, [16], [20]. During pregnancy, progesterone levels fluctuate in waves, peaking at the end of the second trimester and exceeding the value of the first trimester by 29.71% (P<0.05). At the same time, its concentration was higher than in non-pregnant heifers, fluctuating in the range of 21.63-35.63 nmol/l, determining the relevance of the biological effects of progesterone in fetal development.

At the next stage of our work, we tried to assess the dependence of the level of hemostatic parameters in the blood of pregnant and non-pregnant animals on a number of factors, as which the trimester of pregnancy, the level of progesterone in the blood and hemostasis indicators were chosen. Two-factor interactions considered in the pairs "pregnancy trimester x progesterone", "pregnancy trimester x hemostatic indicators", "progesterone x hemostatic indicators", as well as three-factor interaction "pregnancy trimester - progesterone - hemostatic indicators" (Table 3) showed that not only all the main factors are statistically significant, but also their interactions. Consequently, the hemostatic system in the body of heifers /heifer calves depended on the presence of pregnancy and its development, as well as the progesterone level in the blood.

4 Discussion

In different animal species, the level of biochemical and hematological parameters is subject to significant fluctuations during pregnancy, depending on the stage of fetal development, [18], [19], [20], despite the work of homeostatic mechanisms. These variations are caused by an increase in plastic and energy costs due to the provision of embryogenesis needs. Therefore, to control the state of the mother's body, it is important to know the limits of fluctuations in blood parameters and the direction of their changes during pregnancy, which will allow timely detection of any problems of the gestational process.

The results of our study indicate that changes in the hemostasis system occur during pregnancy as its duration and fetal needs increase. This is due to the fact that during pregnancy, the hemostatic systems of the mother and fetus are relatively functionally unrelated, [20].

At the end of the first trimester, the number of platelets, including megalothrombocytes, increased by 19.53 and 13.91% in the blood of pregnant heifers, [3], compared with non-pregnant ones, reflecting both the value of thrombocrit and the volume characteristics of cells. According to, [15], the revealed shifts in the thrombocytogram are associated with the ability of maternal platelets to provide the intervillous lacuna of placenta with proinflammatory mediators, as well as the influence of platelet factors on trophoblasts of the developing placenta and hormone secretion.

During pregnancy, the greatest changes in the thrombocytogram were recorded at the end of the 3rd trimester. At the same time, the development of thrombocytopenia was observed, which is the result of both a decrease in platelet formation, [24], and an increase in plasma volume, the cell consumption level by placenta, their sequestration and circulation, [15]. The revealed changes corresponded to the physiological course of pregnancy, [7], since the direction of the changes in the thrombocytogram was aimed at ensuring the necessary level of blood fluid properties, perfusion of internal organs, metabolic intensity, fetal growth and development rate, as well as the preparation of the mother's body for labor, [25].

The development of pregnancy is also associated with maintaining a balance between the coagulation and anticoagulation systems of the blood, which directly determine the speed of blood flow in the circulation of the mother's body, including the uteroplacental.

Thus, in the context of "pregnant – non-pregnant heifers", [3], there is an "adaptive" increase in blood clotting factors aimed at preventing bleeding during embryo implantation and fetal development. At the same time, the level of indicators characterizing the final stage of blood clotting (thrombin time, fibrinogen) and the external pathway of blood clotting activation (Quick's prothrombin ratio, Quick's prothrombin activity, INR) significantly increases, determining the importance of tissue factors in the blood coagulation potential. In addition, the amount of anticoagulants (antithrombin III) decreases in the blood.

During the pregnancy development, changes in the blood coagulation system are aimed at preparing the mother's body for labor, they follow the hypercoagulation pathway, [26], which is provided, firstly, by a decrease in the concentration of natural anticoagulants (antithrombin III) and an increase in the intensity of fibrin formation processes. According to, [27], this is the result of increased thrombin formation during the preparation of the mother's body for labor.

An important role in pregnancy formation and development is played by the hormone progesterone, which has receptors in the uterus, ovaries, and corpus luteum, [16], [20], [28]. The increase in the concentration of the hormone in the blood of heifers at the onset of pregnancy determines its "locking" effect on the cervix structures, [29], [30]. This contributes to the preservation of pregnancy at an early stage, [31], [32].

During the development of pregnancy, the placenta becomes the main source of progesterone in the mother's body, [16], [20], providing for the needs of both the fetus and the mother. At the same time, progesterone has an indirect effect on the embryo through the endometrium, [31], [32], [33], determining its remodeling potential, [34]. Therefore, during pregnancy, the hormone level in the blood of heifers/heifer calves significantly exceeds the values of non-pregnant animals, [3].

When assessing the dependence of hemostatic blood parameters on progesterone levels and pregnancy trimester, a statistically significant relationship between signs was revealed. Consequently, progesterone participates in the formation of the hypercoagulation state of animals during pregnancy in accordance with its development in trimesters, [16], [20].

5 Conclusions

The hemostasis system during pregnancy of Holstein breed females undergoes significant changes depending on the duration of the gestational process, aimed at creating conditions for fetal development, [35]. The shifts in the thrombocytogram are characterized by a decrease in the number of platelets and thrombocrit by 3.36 and 3.62 times (P<0.05) under conditions of low variability in the average cell volume against the background of an increase in the number of large platelets by 39.87% (P<0.05). The variability of blood clotting tendency parameters is determined not so much by the variability of parameters associated with the activation of the coagulation process, as by a decrease in thrombin time (by 19.88%, P <0.05) and an increase in fibrinogen concentration (by 38.98% (P<0.05), reflecting the potential of the fibrin formation process. This is also facilitated by a decrease in the level of antithrombin III (blood anticoagulant) by 37.04%. In the course of the studies, the average values of the parameters of the thrombocytogram and the blood coagulation system for each trimester of physiologically occurring pregnancy were determined, which can be used to assess the condition of pregnant animals. This will allow timely detection of various abnormalities in the hemostatic system and carry out appropriate preventive measures.

The presence of shifts in the hemostatic system of pregnant calves/heifers during physiological pregnancy determines the need to conduct further research in case of various complications of the gestational process.

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