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Physiological Indices of Platelet-Coagulation Hemostasis in Purebred Ireshire Cows in The Course of Lactation.

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ABSTRACT

Development of all the ontogenesis stages of a cow is closely connected with the dynamics of the functional state of those systems which regulate its body. Blood is among those systems. Hemostasis is physiologically important biological subsystem of blood which provides preservation of its liquid state and stops bleeding. The aim of the research: to study peculiarities of platelet-coagulation hemostasis in healthy Ireshire cows in the course of lactation. There were examined 33 lactating Ireshire cows with the usage of hematological and statistical methods of investigation. The most active aggregation of platelets was noted with ADP and collagen. By the end of lactation it was maximal. Ristomicin aggregation had a reverse direction what indirectly pointed at the decrease of von Willebrand's Factor in animals' blood.Disaggregative capacities of platelets in response to ADP and collagen progressively increased in the course of cows' lactationbut decreased in response to ristomicin. By the middle of lactation Ireshire cows were noted to have some deceleration of prothrombinase and thrombin formation. By its end the cows of this breed had some redundancy of hemocoagulation with simultaneous fortification of fibrin clot. Found dynamics of platelet and coagulation activity can be considered as one of the regulators of lactation process and mechanisms of a cow's preparation to calving.

Keywords: platelets, coagulation hemostasis, fibrin clot, cows, Ireshire breed.

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INTRODUCTION

Development of all the ontogenesis stages of an animal is closely connected with the dynamics of the functional state of those systems which regulate and intergrate its body. Blood is among them [1,2]. Hemostasis is physiologically important biological subsystem of blood which provides preservation of its liquid state, prevents and stops bleeding [3,4]. Hemostasis includes some different components. Platelets [5,6] and hemocoagulation [7,8] are the most significant of them. The efficiency of tissue blood supply, prevention and reduction of hemorrhages, thromboses, ischemia and infarct of organs, protection from dissemination of bacteria and toxins out of lesion focuses through the whole body and so on [9,10] mostly depend on their functional perfection.

Studying of hemostasis indices of productive animals' various species has great signification for the practice of agricultural biology. It is known that these indices are closely connected with animals' somatic characteristics [11] and vital activity processes of the whole body [12,13]. Their planned estimation should help to work out age-specific norms for various indices of hemostasis [14,15]. Cognition of these values will help in early diagnostics of hemostasiopathy at any pathology [16,17]. These researches have especially great significance in respect of high-productive cattle breeds as practical application of their results will provide preservation of cows' productive potential [18,19].

Notwithstanding the doubtless biological signification for high productivity of Ireshire cows, their platelet aggregative capacity and functional activity of hemocoagulation are practically studied very poorly. The present research was planned and conducted in order to fill up the system of physiological knowledges about breed peculiarities of Ireshire cows' lactation.

The aim of the research: to study peculiarities of platelet-coagulation hemostasis in healthy Ireshire cows in the course of lactation.

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 18 March, 1986, and confirmed in Strasbourg on 15 June, 2006).

The study was conducted on 47 cows of Ireshire breed at the farm "PlemzavodMajskyi" in Vologda region (Russia). The animals were examined thrice: at the beginning of lactation (2-3 months after calving with an average daily milk yield of 15.1 kg), in its middle (4-5 month of lactation at a daily milk yield of 21.5 kg) and at the end of lactation (6-7 month of lactation at a value daily average milk yield of 16.4 kg). Only healthy animals were enrolled into observation. There were no complications in the course of their in-calf period.

Blood samples were collected from jugular vein of all the heifers in the morning for studying platelet parameters. Sampling was made into a plastic tube containing 3.8% citrate of sodium dilution in the ratio of blood volumes and citrate of sodium –9:1 [20].

The number of platelets in animals' blood was determined by electron- automatic method on hematological analyzer BC-3000 PLUS (the firm "Shenzhen Mindray Bio-Medical Electronics Co., Ltd.", China).

Platelets' aggregative activity was determined by quantitative method with application of photo-electro-colorimeter KFK-2 (Russia) with such aggregation inductors as ADP, collagen and ristomicin in standard concentrations. Platelets' aggregation was estimated according to the values of summarizing index for platelets' aggregation (SIPA), speed of aggregation (SA) and index of platelets' disaggregation (IPD) [21].

The value of SIPA was found with the help of the formula:

$$SIPA = \frac{E1 - E2}{E1 - E} \times 100\%,$$

where:

E - optical density of rich in platelets plasma in units of optical density;
E1 - optical density of platelet depleted plasma before aggregation in units of optical density;
E2 - optical density of platelet depleted plasma after aggregation in units of optical density.

The value of platelets' aggregation speed was found according to the formula:

$$SA = \frac{E1 - E2}{T},$$

where:

E1 - optical density of platelet depleted plasma before aggregation in units of optical density;
E2 - optical density of platelet depleted plasma after aggregation in units of optical density;
T - period of time, during which maximal fall of optical density took place, in min.

The index value of platelets' disaggregation was calculated according to the formula

$$IPD = \frac{E3 - E2}{E3} \times 100\%$$

where:

E2 - optical density of platelet depleted plasma after aggregation in units of optical density;
E3 - maximal optical density of platelet depleted plasma, measured in 10 minutes after the addition of an aggregation inductor.

The single-channel coagulometer Trombostat (BehnkElektronik, Germany) was used for parameters' determination of plasma-coagulative hemostasis. In all the animals we also determined activated partial thromboplastin time, prothrombin time, thrombin time and concentration of fibrinogen in plasma [21].

In order to conduct thromboflexogram the blood was examined on the analyzer TEG®5000 (Switzerland). While interpreting the received data we took into account five basic parameters of clot formation and its lysis [21]:

R – time of reaction – reflects the speed of thromboplastin formation and corresponds to the first invisible phase of blood coagulation, min.

K – time of coagulation which is necessary for the achievement of maximal clot density, min. Taken together both indices (R+K) characterize the first and the second phases of blood coagulation.

α –angle constant – measures the speed of firm fibrin clot formation (the process of induration or the strength degree of clot formation). The index is espressed in degrees.

MA – maximal amplitude – reflects flexibility (elasticity) of a clot. It corresponds to the third phase of blood coagulation and is expressed in mm. MA value corresponds to the end of coagulation productive phase after which the retraction of a clot and fibrinolysis begin. The index reflects the activity of platelets and fibrinogen.

LY 30 – the index of fibrinolysis – the percent to which the clot volume decreases in the course of 30 minutes after MA achievement.

The received data were processed by Student's t-criterion in the program StatSoft STATISTICA for Windows 6.0.

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RESULTS OF THE RESEARCH

Accountable common platelet indices in the examined animals (the quantity of platelets, the average volume of platelets and thrombokrit) were within the norm and didn't change in the course of observation (Table).

Table: Hemostasis characteristics of cows of Ireshire breed in the course of during lactation

Indicators	Lactation periods, n=33, M±m		
	initial period (1-3 month of lactation)	average period (4-5 month of lactation)	closing period (6-7 month of lactation)
Quantity of platelets, thousand/mcl	358.6±16.70	363.4±13.00	348.1±8.10
Average platelet count, fl	7.2±0.70	7.4±0.20	7.3±0.15
Thrombote, %	0.27±0.03	0.27±0.01	0.27±0.02
Inductor of aggregation ADP			
SIPA, %	27.6±0.64	20.0±0.59**	31.4±0.49**
SA, min	0.048±0.0008	0.023±0.0005**	0.036±0.0002**
IPD, %	14.8±0.85	11.9±0.45*	19.0±0.65**
Inductor of aggregation collagen			
SIPA, %	10.2±0.22	13.3±0.93*	24.0±0.99**
SA, min	0.009±0.0002	0.006±0.0006**	0.018±0.0005**
IPD, %	3.0±0.29	2.4±0.29**	13.7±0.67**
Inductor of aggregation ristomicin			
SIPA, %	12.5±0.42	9.4±0.71*	1.6±0.32**
SA, min	0.009±0.0001	0.007±0.0005*	0.009±0.0006*
IPD, %	3.3±0.09	2.4±0.32**	1.9±0.31**
indicators of coagulation hemostasis			
Fibrinogen, g / l	2.9±0.20	4.2±0.30*	6.3±0.28*
Prothrombin time, s	18.1±0.30	20.8±0.25*	14.1±0.34**
Activated partial thromboplastin time, s	52.1±0.15	59.9±0.83*	47.1±0.29*
Thrombin time, s	16.7±0.30	18.6±0.17*	15.0±0.42*
thromboelastogram			
R, min	20.2±0.69	23.3±0.49*	10.8±0.04**
K, min	8.8±0.48	11.8±0.20**	4.6±0.76**
α, º	30.2±0.20	28.3±0.36	47.5±0.68**
MA, mm	79.4±0.16	76.5±0.35	80.6±0.32
LY30, %	0.01±0.001	0.06±0.005**	0.03±0.003**

Note: p – reliable dynamics of indices was not received.

In the result of the conducted estimation of platelets' aggregative activity in Ireshire cows there were detected reliable differences between the considered periods of lactation (Table). The largest response of platelets was noted to ADP and collagen. At the same time, SIPA with ADP decreased to the middle of lactation till 20.0±0.59% and then increased to its end till 31.4±0.49%. In response to collagen SIPA gradually rose in animals till 24.0±0.99% in the course of lactation. It pointed at the increase of platelets' sensitivity in lactating Ireshire cows to these inductors of aggregation and the intensification of the secretory process out of them under the impact of these substances. The activity of platelets' aggregation under the impact of ristomicin

gradually lowered in the observed cows in the course of lactation – SIPA at its start was equal to 12.5±0.42% reaching to its end 1.6±0.3%.

The speed of aggregates' formation in response to collagen reliably increased in Ireshire cows by the middle of lactation from 0.009±0.0002 min till 0.006±0.0006 min inhibiting to its end. SA had the same dynamics under the impact of ADP and ristomicin. By the middle of cows' lactation it was equal to 0.023±0.0005 min and 0.007±0.0005 min ande by its end – 0.036±0.0002 min and 0.009±0.0006 min, respectively.

Judging by the index value of platelets' disaggregation which shows the stability of appearing aggregants, the most stable aggregants were those ones which were formed in response to ristomicin. IPD value with it gradually lowered in the course of lactation reaching 1.9±0.31%. The aggregates which were formed under the impact of ADP and collagen, were less stable in lactating cows: IPD with them decreased by its middle and increased by its end reaching with collagen 13.7±0.67%, with ADP – till 19.0±0.65%.

The index of activated partial thromboplastin time characterizing the first phase of hemo coagulation, somewhat decelerated in Ireshire cows by the middle of lactation (59.9±0.83s) and then – accelerated. The value of prothrombin time slightly lengthened by the middle of lactation and reliably shortened by its end till 14.1±0.34 s. The state of the third phase of hemocoagulation in Ireshire cows was characterized by two indices: thrombin time and concentration of fibrinogen in plasma. Thrombin time in animals somewhat increased by the middle of lactation (18.6±0.17 s) and then – significantly decreased till 15.0±0.42 s. At the same time, the level of fibrinogen in animals' blood increased in the course of the whole observation reaching by its end 6.3±0.28 g/l.

The time of reaction (R) in the observed cows reflecting the speed of thromboplastin formation, increased by the middle of lactation and then – decreased by its end till 10.8±0.04 min. The index of coagulation time (K) had similar dynamics – increasing by the middle of lactation (11.8±0.20 min) and decreasing by its end (4.6±0.76 min). The value of α index characterizing the strength of a fibrin clot in the observed cows had no significant changes by the middle of lactation. Later it increased reaching by the end of observation 47.5±0.68 degrees.

Physical properties of a clot in Ireshire cows changed insignificantly in the course of the whole lactation having a weak trend to tightening by the end of observation. It was pointed at by the dynamics of the index "Maximal amplitude" which was equal to 79.4±0.16 mm at the start of lactation, in its middle – to 76.5±0.35 mm, and slightly increased by its end till 80.6±0.32 mm. The value of fibrinolysis index (LY 30) was the largest at the peak of lactation and lowered by its end.

DISCUSSION

Modern high level of knowledge about the role of hemostasis in the provision of the internals' functional activity allows considering this system to be especially significant in the maintenance of physiological optimum of the whole body [22]. The activity of hemostasis is variegated in various parts of vascular course. The necessary hemostatic level is established in functionally active organs for the present moment. Sometimes it differs from the same one in the common bloodstream. It is connected with mosaicism of hemostasis system in various parts of vascular course [23].

Last years' researches significantly widened our notions about the factors influencing the aggregation of regular elements [13] and the system of coagulation [24], and also preservation of blood in liquid state. A number of aspects of platelet and coagulative hemostasis components in cattle in the course of ontogenesis remain to be studied rather poorly [25]. Their breed peculiarities (in particular – of Ireshire breed) are still unclear, including the course of dead- lactation periods.

It is known that a cow's body experiences great physiological tension in the course of the whole lactation what causes definite shifts in functioning of all the organs and systems of a body. Just at this period the tissues of udder are mostly subjected to the impact of unfavorable environmental factors and need maximal inflow of blood having good liquid properties [26].

The conducted researches of lactating Ireshire cows detected that the quantity of platelets and their average volume didn't exceed the bounds of conventional normative meanings [16]. At the same time, platelets' aggregative activity in them at different periods of lactation had its own peculiarities. Platelets reacted most actively in response to ADP and collagen. SIPA with these inductors increased with the increase of lactation term. At the same time, SIPA decreased in response to ristomicin. It indirectly pointed at the developing in the course of lactation quantity lowering of von Willebrand's Factor in their blood [25]. The speed of aggregation with all the inductors in the observed animals in the course of lactation decreased at the start and then – increased in different degrees. It pointed at the dynamics of the number of corresponding receptors on platelet membreanes.

Disaggregative capacities of platelets in the course of the whole lactation in response to ristomicin decreased nearly doubly. This index rose in response to ADP and collagen. Given phenomena can be also explained by receptor changes of platelets' membranes and the dynamics of platelet mechanisms of their activation (synthesis of thromboxane, phosphatidic acid and the factor of platelets' activation).

The conducted in the observed cows estimation of separate hemocoagulation indices allowed detecting of their weakening by the middle of lactation and subsequent strengthening by its end at the increase of plasmatic level of fibrinogen. It should be estimated as an important breed mechanism of functioning of blood coagulating system in Ireshire cows which influences the level of the yield of milk in the course of the in-calf state [27,28].

The method of thromboflexography was applied for complex estimation of functioning of hemocoagulative component of the hemostasis system in Ireshire cows. Given method is based on measuring of viscoelastic properties of a blood clot. It allows estimating coagulation by not only determining the kinetics of the start and the end of thrombus formation but also by its stability detecting.

In the result of estimation of the conducted thromboflexogram we can say that the speeds of prothrombinase and thrombin formation lower in Ireshire cows by the middle of lactation. It was pointed at by the increase of R and K indices in them. Developing at that decrease of MA value confirmed the lowering of platelets' aggregative capacity. It becomes clear that achieved hypocoagulation in combination with low platelets' activity can rather positively influence blood rheological properties on the whole. It can optimize blood supply of mammary gland and strengthen milk secretion in the middle of lactation [29,30].

The developing by the end of lactation slightly surplushemocoagulation in Ireshire cows was pointed at by shortening of reaction time (R) and time of coagulation (K) with simultaneous increase of strength of a fibrin clot (α). Given situation in combination with strengthening of platelets' aggregation in response to ADP and collagen can worsen blood rheological properties and rise the level of daily yields of milk.

CONCLUSION

The estimation of productive animals' hemostasis indices closely connected with their somatic characteristics and functioning of the whole body has great practical signification for biology of these animals. It will allow working out of age-specific norms of accountable indices and get criteria of the beginning of hemostasiopathy coming at separate states. In the course of the conducted research we detected the dynamics of indices of platelet and coagulation hemostasis in Ireshire cows in the course of lactation. Low hemocoagulation, active fibrinolysis and low platelets' activity in the middle of it provide optimal conditions for bloode supply of actively working mammary gland promoting the maximal level of the yields of milk. Found dynamics of hemostasis activity in Ireshire cows by the end of lactation is considered to be the regulatorof the yields of milk level and the mechanism of a body's preparation to calving.

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