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Physiological Peculiarities of Platelet-Coagulative Hemostasis in Dead-Wood Cows of Ireshire Breed.

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ABSTRACT

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. The last period of in-calf state in cows has large physiological and productive significance. The aim of the research: to study peculiarities of platelet-coagulative hemostasis in healthy dead-wood cows of Ireshire breed. There were examined 47 dead-wood cows of Ireshire breed with the usage of hematological methods of investigation. The most active platelets' aggregation was noted in response to ADP and ristomicin. Collagenous aggregation was lower what indirectly pointed at low expression of collagenous receptors on the surface of the animals' platelets. Disaggregative platelets' capabilities in response to any inductors progressively increased in cows of Ireshire breed in the course of dead-wood period. In the course of dead-wood period the cows of this breed were noted to have some decrease of hemocoagulation activity at lowering of fibrinogen concentration in their blood.

Keywords: platelets, coagulative hemostasis, fibrin clot, cows, Ireshire breed, dead-wood period.

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INTRODUCTION

The realization of ontogenesis is inseparably connected with physiological dynamics of the systems regulating and integrating living beings [1]. Blood is among them [2,3]. Hemostasis [4,5] is its rather important subsystem providing preservation of its liquid state and reduction of bleedings.

Hemostasis includes some different components. Platelets [6,7] and hemocoagulation [8,9] 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 [10,11] mostly depend on their functional perfection.

The estimation of hemostasis indices of productive animals which are closely connected with their somatic characteristics [12,13] and functioning of the whole body [14,15] has great practical significance for biology. It will allow working out of age-specific norms of accountable indices and determining of the beginning of hemostasiopathy coming at separate states. The in-calf period [16] in cows has a large physiological and productive significance. Studying the cows of Ireshire breed in the course of dead-wood period seems to be very urgent because of their high productivity and great signification of platelet activity and blood coagulation for their somatic status and productivity.

The aim of the research: to study peculiarities of platelet-coagulative hemostasis in healthy dead-wood cows of Ireshire breed.

MATERIALS AND METHODS

All the investigations in the present work were conducted in full correspondence with ethical norms and recommendations on humanization of work with laboratory animals containing "The European Convent on the protection of vertebrate animals used for experiments or in other scientific purposes" (Strasbourg, 1986).

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 dead-wood period (the 1^{st}-2^{nd} days), in the middle of dead-wood period (2^{nd}-31^{st} days) and at the end of dead-wood period (58^{th}-60^{th} days). 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 [17].

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) [18].

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;

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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 coagulometerTrombostat (BehnkElektronik, Germany) was used for parameters' determination of plasma-coagulative hemostasis. In all the animals we also determined activated partial thromboplastintime, prothrombintime, thrombin time and concentration of fibrinogen in plasma [18]. The received deata were processed by Student's t-criterion in the program StatSoft STATISTICA for Windows 6.0.

RESULTS AND DISCUSSION

Common platelet indices of the examined animals 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 dead-wood period

Indicators	Periods of deadwood, n=33, M±m		
	initial period (1-2 day dead-wood)	average period (29-31 day dead-wood)	closing period (58-60day dead-wood)
Quantity of platelets, thousand/mcl	335.4±12.8	341.2±10.62	330.4±7.94
Averageplateletcount,fl	7.3±0.63	7.2±0.42	7.3±0.38
Thrombote, %	0.27±0.05	0.27±0.04	0.27±0.04
Inductor of aggregation ADP			
SIPA, %	30.02±3.42	28.05±2.26	27.22±2.36
SA, min	0.042±0.009	0.044±0.004	0.047±0.003
IPD, %	12.62±1.46	13.2±1.82	14.22±1.28
Inductor of aggregation collagen			
SIPA, %	13.65±1.37	12.10±0.85	10.16±1.37
SA, min	0.0077±0.004	0.0082±0.0005	0.0095±0.0028
IPD, %	2.23±1.34	2.53±1.26	3.00±1.49
Inductor of aggregation ristomicin			

SIPA, %	15.83±1.23	14.22±1.46	12.00±2.61
SA, min	0.0078±0.0062	0.0085±0.0046	0.0095±0.0034
IPD, %	3.11±0.56	3.22±0.44	3.40±0.32
indicatorsofcoagulationhemostasis			
Fibrinogen, g / l	3.12±0.24	3.02±0.27	2.90±0.25
Prothrombintime, s	17.05±0.26	17.82±0.44	18.10±0.27
Activated partial thromboplastin time, s	47.62±0.94	50.14±1.11	52.00±0.98
Thrombintime, s	15.90±1.14	16.20±1.12	16.70±1.26

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

In the result of studying of platelets' aggregative activity in cows of Ireshire breed there were detected reliable differences between separate periods of lactation (Table).

We found a trend to weakening of platelets' aggregation in cows of Ireshire breed in the course of dead-wood period. The strongest response of platelets was noted to ADP and collagen. At the same time, SIPA (summary index of platelets' aggregation) with ADP decreased to the middle of dead-wood period till 28.05±2.26% lowering to its end till 27.22±2.36%. In response to collagen the animals' SIPA gradually lowered in the course of dead-wood period till 10.16±1.37%. It pointed at the lowering of platelets' sensitivity to inductors of aggregation in cows of Ireshire breed in the course of dead-wood period at unexpressed secretory process out of platelets during the activation of plates. The activity of platelets' aggregation in cows of Ireshire breed under the impact of ristomicin gradually lowered in the course of dead-wood period – SIPA at its beginning was equal to 15.83±1.23% reaching to its end 12.00±2.61%.

The speed of aggregates' formation in cows of Ireshire breed in response to collagen lowered in the course of dead-wood period from o.0077±0.004 min till 0.0095±0.0028 min. The same dynamics was experienced by SA (speed of aggregation) under the impact of ADP and ristomicin being equal in cows to the end of dead-wood period to 0.047±0.003 min and 0.0095±0.0034 min, respectively.

The index of platelets' disaggregation showing the resistance of appearing aggregants allowed finding out that the most stable aggregates were those which were formed in response to collagen – the value of IPD (index of platelets' disaggregation) with it reached to the end of dead-wood period 3.00±1.49%. The aggregates which were formed under the impact of ADP and ristomicin, were less stable in the course of dead-wood period: IPD in respect of both increased with ristomicin till 3.40±0.32%, with ADP – till 14.22±1.28% to the end of observation.

The index of activated partial thromboplastin time characterizing the 1st phase of hemocoagulation decelerated a bit in cows of Ireshire breed in the course of dead-wood period (52.00±0.98 s). The activity of the outer way of coagulation in the observed cows was estimated according to the dynamics of prothrombin time which was a bit lengthened in the course of dead-wood period till 18.10±0.27 s. The state of the third phase of hemocoagulation in dead-wood cows of Ireshire breed was characterized by two indices: thrombin time and concentration of fibrinogen in plasma. Thrombin time in the animals increased a bit (16.70±1.26 s). At the same time, the concentration of fibrinogen in blood of animals gradually lowered in the course of observation reaching 2.90±0.25 g/l.

Last years' researches significantly widened our notions about the factors influencing the aggregation of regular elements [19] and the system of coagulation [20], 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 [21]. Their breed peculiarities (in particular – of Ireshire breed) are still unclear, including the course of dead-wood period.

Platelets of dead-wood cows reacted most actively on the impact of ADP and ristomicin. With the increase of dead-wood period SIPA with these inductors lowered. At the same time, SIPA was less in response to collagen. It indirectly showed the low availability of subendothelial collagenfor platelets. The speed of

aggregation lowered in response to all the inductors in the observed animals in the course of dead-wood period what pointed at number lowering of receptors on platelet membranes [22].

Disaggregative capabilities of platelets rose in the course of dead-wood period. It can be considered as the result of receptor displacements of platelets' membranes and physiologically permissible weakening of platelet mechanisms of their activation (synthesis of thromboxane, phosphatidic acid and the factor of platelets' activation) [23].

The conducted in cows of Ireshire breed in the course of dead-wood period estimation of indices characterizing separate mechanisms of hemocoagulation, allowed detecting their weakening in both ways of activation [24] at steady lowering of fibrinogen plasma level. It should be estimated as, first of all, an important breed mechanism of functioning of blood coagulation system in these cows promoting preparation to calving.

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. The last period of in-calf state in cows has large physiological and productive significance. Found in Ireshire cows weakening of hemocoagulation and lowering of platelets' activity at the end of dead-wood period provide optimal conditions for blood supply of placenta and fetus. It also mostly promotes finishing of a body's preparation to calving and strengthening of anabolic processes in it.

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