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Functional Features Of Vascular-Platelet Interactions In Pregnant Cows.

Zavalishina S Yu*.

Russian State Social University, st. V. Pika, 4, Moscow, Russia, 129226.

ABSTRACT

The balance of the functional activity of the components of the primary hemostasis is the basis of the adequacy of the hemostatic process in vivo, and, thus, the optimum liquid properties of blood in the microvasculature and the adequacy of blood supply to all tissues of the animal throughout life. The goal is to find out the physiological features of platelet and vascular hemostasis in healthy cows during normal pregnancy. The study was performed on 47 healthy black-and-white cows, which were examined and examined during pregnancy 7 times: on the day of insemination, on 45, 90, 135, 180, 230 and 280 days of pregnancy. An assessment of the state of vascular-platelet hemostasis in cows after successful insemination indicates the existence of a pattern in the dynamics of their activity of its individual components as the duration of pregnancy increases. During pregnancy, cows showed a weakening of the platelet activity and an increase in the vascular component of hemostasis, providing the necessary fluid properties of blood and, thereby, contributing to the formation of optimal conditions for anabolism in fetal tissues.

Keywords: cows, pregnancy, platelets, vascular wall, antiaggregation.

**Corresponding author*

INTRODUCTION

Blood platelet activity in the blood is normally well balanced by the hemostatic properties of the vessel walls [1-5]. This balance provides the optimum liquid properties of blood [6,7], and, consequently, the adequacy of the level of trophism of their tissues [8-10].

As a result of previous studies, it became known that platelets and the vascular wall in ontogenesis are closely functionally related, determining the aggregative state of the blood [11-13]. The balance of the functional activity of the components of primary hemostasis is the basis of the adequacy of the hemostatic process *in vivo*, and thus the optimum liquid properties of blood in the microvasculature and the adequacy of the blood supply to all animal tissues throughout life [14,15]. Cell hemostatic relationships are most important during pregnancy, when a growing fetus needs intensive oxygen and nutrients, which largely depends on the state of primary hemostasis in the maternal organism [16,17].

A very important economic importance is the comprehensive assessment of vascular-platelet interactions in cattle - an important source of meat and dairy products for the population of Russia and the whole world [18,19]. At the same time, the state of functional readiness of platelets and blood vessels to participate in hemostasis in cows during pregnancy remains very little studied, despite their large role in ensuring the optimum state of the vital systems of the fetus, which is one of the important points of application of corrective action. in animal pathology [20,21]. Considering the extreme physiological and economic importance of the process of bearing offspring in cattle and the weak knowledge of the activity of the primary hemostasis system throughout its length, it was decided to assess the functionality of the platelet and vascular components of hemostasis in healthy cows from insemination to the end of pregnancy.

In this regard, in this paper the goal is formulated: to clarify the physiological features of platelet and vascular hemostasis in healthy cows during normal pregnancy.

MATERIALS AND METHODS

Research was conducted in strict accordance with ethical principles established by the European Convent on protection of the vertebrata used for experimental and other scientific purposes (adopted in Strasbourg March 18, 1986, and confirmed in Strasbourg June 15, 2006) and approved by the local ethic committee of Russian State Social University (Record №12 dated December 3, 2015).

The work was performed on 47 healthy pregnant cows of the black-and-white breed, which were examined and examined 7 times: on the day of insemination for 45, 90, 135, 180, 230 and 280 days of pregnancy.

The level of functional activity of platelet hemostasis in the observed cows was assessed by the state of platelet aggregation (AP) in response to a number of inductors of ADP (0.5×10^{-4} M), collagen (dilution 1: 2 of the main suspension), thrombin (0.125 units/ml), ristomycin (0.8 mg/ml), H_2O_2 (7.3×10^{-3} M), adrenaline (5.0×10^{-6} M) and a combination of inductors: ADP and adrenaline; ADP and collagen; collagen and adrenaline; ADP and thrombin in similar concentrations with a standardized platelet count in the plasma of 200×10^9 platelets.

The state of exchange of endogenous arachidonic acid (AA) in the blood platelets and the functionality of their cyclooxygenase and thromboxane synthetase were determined in three transfer samples with registration of platelet aggregation on a photoelectrocolorimeter.

In the work, a quantitative assessment of the content of ATP and ADP in platelets was carried out, the severity of their secretion under the action of an inducer (collagen) was determined, and the peculiarities of the protein composition of the cytoskeleton of blood plates (actin and myosin) were established.

The functional properties of vascular hemostasis in animals were determined by its antiaggregatory activity of the vascular wall recorded by AP before and after temporary venous occlusion with all inducers and

their combinations when calculating the value of the antiaggregatory index of vessel wall by dividing the duration of AP against the background of venous stagnation by time the occurrence of AP without it.

The research results are processed by the criterion (td) student.

RESULTS OF THE STUDY

In the blood of healthy pregnant cows, normal platelet counts were recorded. At the time of insemination in cows, the time of AP development under the influence of collagen was 22.9±0.16 s, slowing somewhat by the 135th day of pregnancy and subsequently lengthening in addition to its end (27.9±0.19 s). Similar AP dynamics in the observed animals during pregnancy was noted under the influence of ADP (increased by 15.1%) and ristomycin (increased by 13.9%), thrombin developed slightly (increased by 11.3%) and adrenaline (increased by 7.5%) AP. Platelet aggregation with all the tested physiological combinations of inductors in the observed pregnant animals also gradually slowed down compared with indicators characteristic of the moment of insemination (Table).

Table. Indicators of vascular platelet hemostasis in pregnant cows

Taken into account indicator	Pregnancy, n=47, M±m						
	insemination	45 day	90 day	135 day	180 day	230 day	280 day
Aggregation with ADP, s	31.9±0.18	32.0±0.22	32.8±0.20	33.8±0.19	35.0±0.23	36.1±0.26	37.6±0.24
Aggregation with collagen, s	22.9±0.16	23.0±0.14	23.6±0.18	24.8±0.21	25.7±0.24	26.8±0.23	27.9±0.19
Aggregation with thrombin, s	44.6±0.12	44.7±0.20	45.1±0.16	46.1±0.27	47.7±0.29	49.0±0.31	50.3±0.26
Aggregation with ristomycin, s	39.6±0.19	39.8±0.15	40.6±0.21	41.8±0.24	42.7±0.28	44.3±0.23 p<0.05	46.0±0.24 p<0.05
Aggregation with H ₂ O ₂ , s	33.2±0.18	33.4±0.16	34.6±0.14	35.9±0.17	37.1±0.23	38.6±0.21 p<0.05	39.8±0.27 p<0.05
Aggregation with adrenaline, s	86.4±0.31	86.7±0.27	87.5±0.25	89.2±0.29 p<0.05	90.6±0.32	91.4±0.35	93.4±0.37 p<0.05
Aggregation with ADP and adrenaline, s	29.2±0.16	29.4±0.17	30.4±0.12	31.7±0.15	32.8±0.17	33.9±0.12	35.6±0.19 p<0.05
Aggregation with ADP and collagen, s	20.4±0.12	20.5±0.14	21.4±0.16	22.3±0.09	23.4±0.13	24.5±0.15	25.7±0.23
Aggregation with adrenaline and collagen, s	21.2±0.11	21.3±0.17	22.6±0.22	23.5±0.18	24.4±0.15	25.6±0.20	27.2±0.18 p<0.05
Aggregation with ADP and thrombin, s	20.7±0.12	20.8±0.15	21.6±0.13	23.2±0.15	24.6±0.17	26.0±0.22 p<0.05	28.1±0.14 p<0.05
index of antiaggregatory activity of the vessel wall with ADP	1.89±0.12	1.89±0.08	1.91±0.06	1.93±0.07	1.94±0.09	1.96±0.05	1.98±0.08
index of antiaggregatory activity of the vessel wall with collagen	1.77±0.06	1.77±0.04	1.78±0.07	1.80±0.05	1.82±0.09	1.84±0.07	1.87±0.04 p<0.05
index of antiaggregatory activity of the vessel wall with thrombin	1.64±0.10	1.64±0.07	1.65±0.08	1.67±0.09	1.69±0.10	1.71±0.08	1.74±0.12 p<0.05

index of antiaggregatory activity of the vessel wall with ristomycin	1.66±0.06	1.67±0.04	1.68±0.07	1.70±0.04	1.72±0.07	1.74±0.09	1.77±0.06 p<0.05
index of antiaggregatory activity of the vessel wall with H ₂ O ₂	1.75±0.10	1.76±0.12	1.78±0.06	1.81±0.07 p<0.05	1.84±0.04 p<0.05	1.87±0.08 p<0.05	1.91±0.11 p<0.05
index of antiaggregatory activity of the vessel wall with adrenaline	1.77±0.11	1.78±0.06	1.80±0.08	1.83±0.07 p<0.05	1.86±0.05 p<0.05	1.90±0.06 p<0.05	1.93±0.09 p<0.05
index of antiaggregatory activity of the vessel wall ADP and adrenaline	1.58±0.03	1.59±0.05	1.62±0.04 p<0.05	1.64±0.08	1.67±0.10 p<0.05	1.70±0.09 p<0.05	1.74±0.07 p<0.05
index of antiaggregatory activity of the vessel wall ADP and collagen	1.49±0.10	1.50±0.06	1.53±0.08 p<0.05	1.54±0.08	1.56±0.09	1.59±0.10 p<0.05	1.61±0.12
index of antiaggregatory activity of the vessel wall adrenaline and collagen	1.62±0.12	1.62±0.07	1.64±0.08	1.66±0.05	1.68±0.04	1.70±0.05	1.73±0.10 p<0.05
index of antiaggregatory activity of the vessel wall ADP and thrombin	1.49±0.05	1.49±0.03	1.52±0.10	1.54±0.06	1.56±0.08	1.59±0.07 p<0.05	1.63±0.10 p<0.05

Legend: p - accuracy of the age dynamics of the indicators taken into account.

A serious mechanism for the inhibition of platelet aggregation in pregnant cows is a pronounced weakening of the intensity of arachidonic acid exchange in blood plates with a gradual decrease in thromboxane formation, which could be indirectly judged by AP in a simple transfer test (decrease by 29.0%). This dynamics was provided by a gradual simultaneous weakening of the activity of both enzymes of its conversion in platelets - cyclooxygenase and thromboxide dismutase. The degree of AP recovery in the collagen-aspirin test, which indirectly assessed the activity of cyclooxygenase in platelets, decreased during pregnancy by 8.2%, reaching 81.8±0.36% by the end of pregnancy. The recovery of antibodies in the collagen-imidazole sample, which indirectly determines the functional activity of thromboxide dismutase in blood plates, gradually decreased in observed cows by 18.1%, reaching 42.6±0.17% for 280 days of pregnancy.

The initially low content of ATP and ADP in the platelets of healthy cows gradually decreased during pregnancy from 5.87±0.07 μmol/10⁹ platelets to 5.58±0.13 μmol/10⁹ platelets and from 3.71±0.11 μmol/10⁹ platelets to 3.39±0.15 μmol/10⁹ platelets, respectively. At the same time, their level of secretion from platelets experienced a similar trend, decreasing from 42.7±0.26% and 52.7±0.24% at the time of insemination to 37.6±0.24% and 47.8±0.23% by the end of pregnancy, respectively.

The amount of actin and myosin in intact platelets in cows at the time of insemination was 37.8±0.19% and 18.5±0.12% of the total protein in platelets, gradually decreasing to 280 days of pregnancy to 32.6±0.25 % and 16.3±0.09% of total protein in platelets.

In healthy cows monitored during pregnancy, increased control of the vascular wall over AP was revealed. The highest index of antiaggregation activity of the vessel wall was recorded with ADP. A slightly lower level of the antiaggregation index of the vessel wall was detected for collagen and adrenaline. The index of antiaggregatory activity of the vessel wall for thrombin and ristomycin was even lower in absolute values. Rather high index of antiaggregatory activity of the vessel wall for combinations of inductors also experienced a tendency to increase during pregnancy (table).

DISCUSSION

A very biologically significant integrative system of the body of cattle is the blood system, the fluid properties of which are regulated by hemostasis [18,19]. The rheological properties of blood and, thus, the optimum growth and development of the fetus [20] largely depend on its optimal activity during pregnancy. At the same time, the analysis of the level of activity of platelet, vascular and coagulation hemostasis and the subtle mechanisms for their implementation in healthy pregnant cows are not well understood.

Estimation of the time of platelet aggregation under the influence of a number of inductors and a large number of their physiological combinations made it possible to determine from the moment of insemination for 280 days of pregnancy in cows a weakening of the sensitivity of platelets to them. It has been found that the adhesive ability of the platelets is experiencing a similar dynamic, probably due to a decrease in the concentration of von Willebrand factor in their blood - a platelet adhesion cofactor, which apparently combines with a decrease in the number of receptors for it - (GPI) on the surface of blood plates [21]. The dynamics of von Willebrand factor in pregnant cows was established on the basis of gradual inhibition of platelet aggregation with ristomycin, which, by its ability to influence platelets, is identical to the subendothelial structures of blood vessels [22]. Attaching one end of the molecule to the collagen, and the other to the platelet via the glycoprotein Ib, von Willebrand factor forms the "adhesion axis": collagen – von Willebrand factor – GPIb [23]. This suggests a decrease in the number of these receptors on the platelet membranes in cows during pregnancy.

Attenuation of expression of platelet sensitivity to various agonists aggregation and their combinations likely also associated with a reduced expression of the fibrinogen receptor (GPIIb-IIIa), the level of stimulation of phospholipases A₂ and C, intensities thromboxanoformation synthesis of actin and myosin adenosine phosphates and secretion of platelets during pregnancy [24, 25].

The revealed tendency to enhance the antiaggregation activity of the vascular wall in pregnant cows is explained by the increased synthesis of prostacyclin and NO in it, providing the necessary level of microcirculation in the tissues of the placenta [26,27].

CONCLUSION

An assessment of the state of vascular-platelet hemostasis in cows after successful insemination indicates the existence of a pattern in the dynamics of their activity of its individual components as the duration of pregnancy increases. Thus, during pregnancy, cows have a weakened platelet activity and increased vascular component of hemostasis, providing the necessary fluid properties of blood and, thus, contributing to the formation of optimal conditions for anabolism in fetal tissues.

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