

VASCULAR-PLATELET INTERACTION IN PREGNANT COWS

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Abstract

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Balance of components' activity of thrombocyte-vessel hemostasis is the basis of the whole hemostasis process functioning in conditions of 'in vivo' and, thus, optimum of fluidic blood features and adequacy of blood supply of all the tissues of an animal during whole its life. The aim is – to find out physiological peculiarities of thrombocyte-vessel hemostasis of healthy cows during the normal process of pregnancy. The investigation was fulfilled with the help of 47 healthy cows of black-variegated breed which were examined and investigated 7 times during pregnancy: on the day of insemination, on the 45th, 90th, 135th, 180th, 230th and 280th day of pregnancy. The received results of state evaluation of thrombocyte-vessel hemostasis of cows after successful insemination testify to the existence of physiological regularity in the dynamics of its separate components' activity. It was found that during pregnancy the cows were noted to have weakening of thrombocyte hemostasis component's activity and strengthening of vessel hemostasis component. It provides them with necessary hemostatic and fluidic blood features, thus promoting the formation of optimal conditions for placenta blood supply and anabolism in fetus' tissues.

Key words: cows, pregnancy, thrombocytes, vessel wall, hemostasis

Introduction

After some investigations fulfilled earlier it became clear that thrombocytes and vessel wall in ontogenesis are closely functionally interconnected (Shitikova, 2008). They mostly define the aggregate normal blood state and pathology of a man (Medvedev and Zavalishina, 2016; Simonenko, et al., 2010) and different kinds of productive animals including cattle (Zavalishina, 2011; 2012; 2013). Balance of functional activity of the initial hemostasis' components is the basis for adequacy of the whole hemostatic process in conditions of 'in vivo', optimum of fluidic blood features in microcirculatory channel and blood supply adequacy in animal's tissues during whole its life (Kutafina and Zavalishina, 2012; Kutafina, 2015). Many physiological and pathological processes are accompanied by changes of thrombocyte and vessel activity what finally influences the state of metabolism in tissues (Medvedev et al., 2016; Shitikova, 2008).

Detailed cattle investigation has great economic meaning as it is the main source of meat-milk production for sig-

nificant part of the planet's population (Gergovska, et al., 2011; Gunay et al., 2011; Kulig, et al., 2016). Because of the close connection of blood state indices and cattle somatic status (Korepanova et al., 2015) it is interesting to investigate hematologic, including hemastatic, indices at different functional states and in different age groups. The initial hemostasis gets the greatest physiological significance during pregnancy when its state in mother's organism provides necessary hemoreological conditions for fetus's supply with oxygen and nutrients (Medvedev and Krasnova, 2014). At the same time peculiarities of thrombocytes' and vessels' functional readiness to the participation in hemostasis processes of cows during pregnancy were not practically investigated before. Shutoff of the given gap in scientific works is able to give great practical run-out as thrombocyte-vessel hemostasis starts to be looked at as one of important application points of correcting influence in case of pathology formation (Belova and Zavalishina, 2011; Medvedev, et al., 2010). Taking into consideration high biological and economical importance of cattle calf-bearing process and the fact that

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activity of thrombocyte-vessel interaction in the system of hemostasis during its continuity was not studied enough, we managed to formulate the aim of present investigation: to find out physiological peculiarities of thrombocyte-vessel hemostasis of healthy cows during the normal process of pregnancy.

Materials and Methods

The work was made with the help of 47 healthy cows of black-variegated breed of the second or third pregnancy which were examined and investigated 7 times: on the day of insemination, on the 45th, 90th, 135th, 180th, 230th and 280th day of pregnancy.

With the help of enzymoimmunoassay method there were defined plasma concentrations of P-selectin molecules and PECAM-1 molecules (Bender MedSystems GmbH, Austria).

Cows under investigation by micromethod were evaluated from the point of view of thrombocytes' aggregation (Medvedev, 2016) in response to some inductors: ADP (0.5×10^{-4} M), collagen (dilution 1:2 of the basic suspension), thrombin (0.125 units/ml.), rystomicin (0.8 mg/ml, H_2O_2 (7.3×10^{-3} M), adrenalin (5.0×10^{-6} M) and combination of inductors: ADP and adrenalin; ADP and collagen; collagen and adrenalin; ADP and thrombin in analogical concentrations with standardized quantity of thrombocytes in the investigated plasma 200×10^9 tr.

The exchange of endogenous arachidonic acid (AA) in thrombocytes and the activity of their enzymes – cyclooxygenaza and thromboxansynthetaza – were defined in three transfer tests by registration of thrombocytes' aggregation in photoelectric colorimeter (Ermolaeva et al., 1992).

In cows' thrombocytes there was fulfilled quantitative evaluation of ATP and ADP content, we also found out the evidence of their secretion in response to collagen and determined the state of protein content of thrombocyte cytoskeleton (actin and myosin content) (Ermolaeva et al., 1992).

Antiaggregative features of animals' vessel wall were defined during registration of thrombocytes' aggregation in blood plasma received before temporal venous occlusion and after it with all the inductors and their combinations. We also made the calculation of indices' meanings of antiaggregative vessel wall activity (IAAVW) by dividing thrombocytes aggregation duration on the background of venous standstill in the period of establishment of thrombocyte aggregation without it for all the inductors (Baluda et al., 1987). Investigation results were processed by Student's criterion (td).

Results

During pregnancy cows' blood is noted to have gradual lowering of concentration of registered adherence molecules (Table 1). During the whole pregnancy level lowering of P-selectin and PECAM-1 turned out to be trustworthy and gave 16.1% and 18.8% correspondingly.

At the moment of cows' insemination the time of thrombocyte aggregation development under collagen influence was equal to 22.9 ± 0.16 s, a bit reducing to the 135th day of pregnancy and then additionally reducing (27.9 ± 0.19 s). The same thrombocyte aggregation dynamics of investigated animals was noted under ADP influence (summarily lengthened on 15.1%), rystomicin (summarily lengthened on 13.9%), thrombin (summarily lengthened on 11.3%) and adrenalin (summarily lengthened on 7.5%). Thrombocyte aggregation with all the used physiological inductors' combinations of observed pregnant cows also gradually lengthened in comparison with indices typical for insemination moment (Table 1).

The important mechanism realizing retardation process of thrombocyte aggregation of pregnant cows is found in their organisms frank intensity reduction of AA exchange in blood platelets with gradual reduction of thromboxanformation in them. It was indirectly judged by retardation of thrombocyte aggregation in simple transfer test (lowering on 29.0%). Given dynamics was provided by gradual simultaneous reduction of both enzymes' activity of transformation in thrombocytes – cyclooxygenaza and thromboxansynthetaza. Inflammation degree of thrombocyte aggregation in collagen-aspirin test indirectly evaluating cyclooxygenaza activity in thrombocytes, during pregnancy reduced on 8.2%, being to its end – $81.8 \pm 0.36\%$. Inflammation of thrombocyte aggregation in collagen-imydazol test making indirectly possible to define functional thromboxansynthetaza activity in blood platelets, in case of observed cows gradually lowered on 18.1% and reached $42.6 \pm 0.17\%$ on the 280th day of pregnancy.

Initially not high content of ATP and ADP in cows' thrombocytes gradually reduced during pregnancy from 5.87 ± 0.07 mkmol/ 10^9 tr. to 5.58 ± 0.13 mkmol/ 10^9 tr. and from 3.71 ± 0.11 mkmol/ 10^9 tr. to 3.39 ± 0.15 mkmol/ 10^9 tr., correspondingly. At the same time their level of secretion from thrombocytes experienced analogical dynamics lowering from $42.7 \pm 0.26\%$ and $52.7 \pm 0.24\%$ at the moment of insemination to $37.6 \pm 0.24\%$ and $47.8 \pm 0.23\%$ to the end of pregnancy, correspondingly.

The quantity of actin and myosin in cows' intact thrombocytes at the moment of insemination was $37.8 \pm 0.19\%$ and $18.5 \pm 0.12\%$ to common protein in thrombocytes gradually

Table 1
Platelet and vascular parameters in pregnant cows

| Consider indicators | Pregnant cows, n = 47, M±m | | | | | | |
|---|----------------------------|------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| | Insemination | 45 hours | 90 hours | 135 hours | 180 hours | 230 hours | 280 hours |
| P-selectin, ng/ml | 108.3±0.48 | 105.8±0.50 | 102.5±0.62 | 100.0±0.56 | 97.1±0.68 | 95.2±0.55 | 93.3±0.42 |
| PECAM-1, ng/ml | 54.2±0.27 | 52.4±0.32 | 56.8±0.35 | 49.7±0.29 | 48.0±0.26 | 46.8±0.31 | 45.6±0.25 |
| platelet 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 |
| platelet 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 |
| platelet 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 |
| platelet aggregation with rystomicin, 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 |
| platelet 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 |
| platelet aggregation with adrenalin, 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 |
| platelet 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 |
| platelet 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 |
| platelet 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 |
| platelet 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 |
| IAAVW 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 |
| IAAVW 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 |
| IAAVW 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 |
| IAAVW with rystomicin | 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 |
| IAAVW 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 |
| IAAVW with adrenalin | 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 |
| IAAVW with 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 |
| IAAVW with 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 |
| IAAVW with 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 |
| IAAVW with 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 – the accuracy of the dynamics of indicators

lowering to the 280th day of pregnancy to $32.6 \pm 0.25\%$ and $16.3 \pm 0.09\%$ to common protein in thrombocytes.

The observed cows during pregnancy were found to have increasing control of a vessel wall over thrombocyte aggregation. It became evident in the increase of IAAVW meanings for all the used inductors and their combinations. The highest IAAVW was registered for ADP. A bit lower IAAVW level was noted for collagen and adrenalin. IAAVW meanings for thrombin and rystomicin in absolute meanings were much lower. Inferior to IAAVW meanings for separate inductors, IAAVW values for their combinations also experienced gradual increase during pregnancy (Table 1).

Discussion

Continuous work aimed at the increase of cattle productivity in the whole world dictates the necessity of planned conducting of physiological investigations able to give necessary information for it (Yavuz, et al., 2015; Yilmaz and Koc, 2013). It is considered that blood system is biologically very meaningful integrative system of cattle's organism (Glagoleva, 2015). Its fluidic features are directly defined by animal's genetic programme (Amelina and Medvedev, 2009) and are mostly regulated by hemostasis (Glagoleva, 2015a,b). Exactly from its optimal activity during pregnancy supply levels of oxygen and nutrients to growing fetus' tissues mostly depend (Medvedev and Krasnova, 2014). Being very meaningful for all the hemostasis stages, thrombocytes' aggregative features and disaggregative vessels' possibilities experience in case of different biological objects characteristic ontogenetic dynamics (Kutafina and Medvedev, 2015) which needs detailed investigation in case of pregnant cows.

Very sensitive indices of thrombocyte-vessel interaction are concentrations in blood of cell adherence molecules of P-selectin and PECAM-1 which have thrombocyte and endothelial origin. Their level points at their expression evidence and thereby, at the potential of thrombocytes and endothelium interaction. It allows considering them as indices characterizing the potential of thrombocyte adherence to endothelium. Besides, it is agreed that levels of concentrations of P-selectin and PECAM-1 interact with changes of thrombocyte aggregation and vessel disaggregative impacts. Proved interaction of cell expression of adherence molecules and their plasma level allows considering concentration lowering of the latter as the reflection of its reduction [19]. Therefore, found in pregnant cows habitude to gradual lowering of circulating P-selectin and PECAM-1 points at risk minimization of blocking episodes of capillary course by microthrombs and provision of optimal conditions for metabolism.

Performed evaluation of thrombocyte aggregation time under the influence of some inductors and their physiological combinations allows ascertaining from insemination moment to the 280th day of cows' pregnancy gradual reduction of thrombocyte sensitiveness to them. Taking into consideration gradual time growing of thrombocyte aggregation on collagen and rystomicin, we can speak about retardation of their blood platelets' adhesive ability. May be, it is founded on concentration lowering of Willybrand's factor in their blood – cofactor of thrombocyte adherence and decrease of receptors' number to it – (GPIb) and to collagen on the surface of blood platelets (Medvedev and Zavalishina, 2016). Gradual lowering of FW level in cows' blood and receptors' density to it on their thrombocytes while the period of pregnancy became longer was established on the basis of retardation increasing of thrombocyte aggregation with rystomicin which, as far as its ability to influence thrombocytes is concerned, is identical to vessels' subendothelial structures. Sticking by one molecule side to collagen and the other – to thrombocyte through glycoproteid Ib, FW forms “adherence axis”: collagen – FW – GPIb (Shitikova, 2008). It also allows speaking about decrease of these receptors' number on thrombocyte membranes in case of cows during pregnancy.

Evidence weakening of thrombocytes' sensitivity to different aggregation agonists and their combinations during pregnancy may also be connected with expression decrease on their surface of fibrinogenic receptors (GPIIb-IIIa), activity decrease of phospholipase A₂ and C, intensity decrease of thromboxanformation, aktin- and myosinformation and secretion of adenosine phosphate from blood platelets (Zavalishina, 2012).

Found tendency to the increase of antiaggregation activity of pregnant cows' vessel wall can be explained by the increase of synthesis of prostacyclin and NO in it (Glagoleva, 2015b; Kutafina and Zavalishina, 2012), providing the necessary microcirculation level in placenta's tissues.

Conclusion

With the advance of pregnancy period cows are registered to have gradual decrease of thrombocyte activity and increase of vessel control over it. Gradual concentration lowering of P-selectin and PECAM-1 circulating in cows' blood with the advance of pregnancy period is an important mechanism of physiological evidence decrease of thrombocytes and endothelium interaction. Given dynamics of thrombocyte-vessel interaction of pregnant cows can be mostly connected with quantity decrease of receptors to aggregation inductors on thrombocytes' surface and production increase of prostacyclin and NO in vessels.

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