

Functional Platelet Activity in Dutch Newborn Calves

Nadezhda V. Vorobyeva^{1,2} and Ilya N. Medvedev³

¹South-West State University, 305040, Kursk, Russia

²All-Russian Research Institute of Physiology, Biochemistry and Nutrition of Animals,
Institute of Village, 249013, Borovsk, Russia

³Russian State Social University, 129226, Moscow, Russia

ABSTRACT

The hemostatic properties of platelets in cattle are physiologically very significant, as they affect the course of metabolic processes. This is especially significant in early ontogenesis and, apparently, strongly depends on the genetic characteristics of animals. The study was carried out on 37 Dutch calves, which were obtained from healthy cows after a normal pregnancy. All calves were examined and examined for 1-2 days, 3-4 days, 5-6 days, 7-8 days and 9-10 days of their ontogenesis. In the work were applied biochemical, hematological and statistical research methods. In the examined animals, during the neonatal phase, there was a tendency to inhibition of platelet aggregation in response to all inductors used. The number of platelet-discocytes in the blood of the examined Dutch calves in the first 10 days of their life experienced an upward trend. The amount of active platelet species they had decreased by 11.1%. The number of small and large platelet aggregates in the blood also decreased in them during the observation period. This was provided in the observed calves with a tendency to weaken the severity of synthesis in thromboxane platelets, a decrease in the level of adenosine phosphates in them and a weakening of their secretion. The level of platelet actin and myosin on the 1-2nd day in the examined calves was small and tended to decrease during the observation. Additional self-assembly of actin and myosin during platelet aggregation of the observed animals experienced some decrease during the observation period. It is clear that newborn calves of the Dutch breed are characterized by a high degree of functional sufficiency of platelets, creating physiologically favorable conditions for microcirculation processes. At the basis of these changes they have a small activity of the mechanisms that implement the hemostatic properties of platelets. Low intravascular activity of platelets in newborn calves of the Dutch breed is able to provide them with optimum perfusion and metabolic processes in all animal tissues that are necessary for the normal development of animals.

KEY WORDS: CALVES, NEWBORN PHASE, DUTCH BREED, PLATELETS, AGGREGATION, SECRETION.

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*Corresponding Author: ilmedv1@yandex.ru

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INTRODUCTION

Hemostatic mechanisms are an important component of maintaining homeostasis (Zavalishina, (2018h). It is clear that the state of the functional indicators of the hemostasis system very significantly determines the processes of hemocirculation (Usha et al., 2019). It is greatly influenced by platelets, the state of activity of which is able to regulate the course of microcirculation (Zavalishina, 2018d) in various living organisms (Kulikov et al., 2019). It is known that hemostatically significant manifestations of platelets are capable of experiencing dynamics at different ages (Zavalishina, 2018a), under the conditions of the formation of many dysfunctions (Chinarov, 2018), the development of pathological processes (Zavalishina et al., 2019) and against the background of their correction by any method (Zuev et al., 2006). However, many aspects of platelet hemostasis in cattle remain poorly understood. At present, only certain facts are known on platelet activity in these productive animals that are at separate stages of their ontogenesis (Vorobyeva et al., 2018).

There is still no complete picture of the relationship between the genetic characteristics of cattle and their platelet activity at different stages of their ontogenesis. At the same time, the efficiency of capillary blood flow, and, consequently, the level of trophism of tissues and the growth rate of all body structures and the formation of their functional characteristics (Sharkayeva et al., 2016; Zavalishina, 2018j), is largely related to the level of platelet activity in calves. Considering the presence of genetic differences between the breeds of cattle, it was of great interest to find out the peculiarities of platelet activity in calves of highly productive in terms of the volume of Dutch milk yield at the very beginning of their ontogenesis - during the neonatality phase. The goal of the present work is to find out the level of platelet activity in Dutch calves during the neonatal phase.

MATERIALS AND METHODS

Research was conducted in strict accordance with ethical principles established by the European Convention 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). The work was performed on 37 calves of the Dutch breed. All calves taken into the study were obtained from healthy cows after normal pregnancy. All calves were examined and examined during the neonatal phase 5 times: 1-2, 3-4, 5-6, 7-8 and 9-10 days of life. All animals underwent an indirect assessment of the activity of synthesis in thromboxane platelets and an indirect elucidation of the enzymatic activity of cyclooxygenase and thromboxane synthetase in them using three transfer tests that evaluated platelet aggregation on a photoelectrocolorimeter. In platelets, the amount of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) was evaluated, as well as the degree of their secretion in response to the contact of collagen with platelets.

The content of actin and myosin in inactive platelets and platelets that have aggregated in response to ADP has been determined (Ermolaeva et al., 1992). The expression of platelet aggregation (AP) was determined using a visual micromethod using as inducers ADP (at a dose of 0.5×10^{-4} M), collagen (at a dilution of 1: 2 from the main suspension), thrombin (at a dose of 0.125 u/ml), adrenaline (at a dose of 5.0×10^{-6} M) and ristomycin (at a dose of 0.8 mg/ml) in plasma which was previously standardized by the number of platelets to the level of 200×10^9 platelets per liter (Shitikova, 2000). The intravascular activity of platelets was determined by applying the method of phase-contrast microscopy (Shitikova, 2000). Statistical processing of received information was made with the help of a programme packet "Statistics for Windows v. 6.0", "MicrosoftExcel". Differences in data were considered reliable in case $P < 0,05$.

RESULTS AND DISCUSSION

The Dutch calves taken under observation during the neonatal period showed a tendency to decrease the initial small platelet activity. Thus, in the examined calves, at 1-2 days of life, the AP developed in response to collagen for 37.4 ± 0.13 s, in subsequent periods of observation it was inhibited, reaching by 9-10 days of life 38.6 ± 0.15 s. A similar trend to slowing down the AP process was found in relation to ADP and ristomycin, which occurred at the end of observation at 47.6 ± 0.26 s and 56.9 ± 0.24 s, respectively. The tendency to slow the development of antibodies in response to thrombin (up to 60.1 ± 0.20 s) and adrenaline (up to 108.2 ± 0.15 s) has also been clarified.

The level of platelet-discocytes in the blood of the examined calves during the neonatal phase has undergone a tendency to increase. During the observation period, the sum of the activated platelet varieties experienced a slight decrease, totaling 11.1%. The number of small and large platelet aggregates in blood in the observed animals in the first 10 days of life gradually decreased by 21.7% and 2 times, respectively. It is clear that the inhibition of AP in Dutch calves during the observation period was largely due to the weakening of their synthesis in thromboxane platelets, which was indirectly indicated by a decrease in AP in a simple transfer test, the rate of which for 9-10 days of life was $24.8 \pm 0.16\%$. These results were provided in the observed calves due to the tendency to a decrease in the activity of both platelet enzymes for the synthesis of thromboxane, cyclooxygenase and thromboxane synthetase. The intensity of AP recovery during the collagen-aspirin test, which characterizes the level of activity in platelets of cyclooxygenase, was $73.0 \pm 0.09\%$ by the end of the observation. The degree of AP recovery in the process of carrying out a collagen-imidazole sample, which makes it possible to indirectly estimate the level of platelet thromboxane synthetase activity, in the examined calves also decreased during the observation time and reached $34.3 \pm 0.12\%$ on day 9-10.

Table. State of platelet activity in newborn calves of Dutch breed

Considered indicators	Calves of dutch breed, n=37, M±m				
	1-2 day	3-4 day	5-6 day	7-8 day	9-10 day
The level of platelet % aggregation recovery during the collagen-aspirin test,	75.9±0.14	75.5±0.17	74.8±0.09	73.7±0.08	73.0±0.09
The level of recovery of platelet aggregation during the collagen-imidazole test, %	36.6±0.11	36.0±0.05	35.6±0.08	35.0±0.07	34.3±0.12
The state of platelet aggregation in a simple transfer test, %	26.5±0.14	26.2±0.12	25.6±0.10	25.0±0.09	24.8±0.16
The amount of ATP in platelets prior to the start of secretion, μmol /109 platelets	5.35±0.018	5.30±0.012	5.26±0.016	5.24±0.007	5.19±0.005
The number of ADP in platelets before the start of secretion, μmol/109 platelets	3.20±0.004	3.16±0.002	3.13±0.007	3.10±0.006	3.07±0.008
secretion level ATP,%	25.5±0.10	25.3±0.12	25.0 ±0.08	24.7±0.07	24.2±0.13
secretion level ADP,%	32.8±0.08	32.6±0.11	32.3±0.07	32.0±0.13	31.5±0.10
The amount of actin in inactive platelets,% of total protein in platelets	20.9±0.14	20.6±0.10	20.0±0.05	19.7±0.06	19.3±0.04
The amount of actin in platelets with ADP-aggregation,% of total protein in platelets	32.4±0.12	32.1±0.06	31.8±0.08	31.5±0.05	31.2±0.10
The amount of myosin in inactive platelets,% of total protein in platelets	9.8±0.15	9.6±0.18	9.4±0.06	9.1±0.07	8.9±0.14 p<0.05
The amount of myosin in platelets with ADP-aggregation,% of total protein in platelets	22.3±0.10	22.1±0.16	21.7±0.05	21.5±0.10	21.2±0.15
Platelet aggregation time with ADP, s	46.7±0.20	46.8±0.17	47.0±0.24	47.3±0.18	47.6±0.26
Platelet aggregation time with collagen, s	37.4±0.13	37.7±0.20	38.0±0.14	38.3±0.19	38.6±0.15
Platelet aggregation time with thrombin, s	58.5±0.12	58.8±0.24	59.4±0.28	59.8±0.17	60.1±0.20
Platelet aggregation time with ristomycin, s	54.6±0.10	54.9±0.17	55.3±0.30	56.6±0.19	56.9±0.24
Platelet aggregation time with adrenaline, s	105.8±0.25	106.8±0.22	107.3±0.16	107.6±0.18	108.2±0.15
The number of platelet platelets, %	84.0±0.21	84.4±0.29	84.9±0.17	85.3±0.23	85.6±0.26
Total Active Platelet Count, %	16.0±0.19	15.6±0.12	15.1±0.16	14.7±0.15 p<0.05	14.4±0.14 p<0.05
The number of small platelet aggregates per 100 free platelets	2.8±0.09	2.6±0.05	2.4±0.03 p<0.05	2.3±0.04 p<0.01	2.3±0.06 p<0.01
The number of medium and large platelet aggregates per 100 free platelets	0.10±0.017	0.09±0.012 p<0.05	0.07±0.014 p<0.01	0.06±0.010 p<0.01	0.05±0.018 p<0.01

Note: p - reliability of the dynamics of indicators in relation to 1-2 daily age

Initially, a small amount of ATP and ADP in the platelets of calves in the observation process experienced a tendency to decrease, reaching 5.19 ± 0.005 and 3.07 ± 0.008 $\mu\text{mol}/10^9$ platelets towards its end. Moreover, during the observation, the levels of their secretion from the platelet granules tended to decrease by 5.4% and 4.1%, reaching $24.2 \pm 0.13\%$ and $31.5 \pm 0.10\%$ by the end of the observation, respectively. In inactive platelets of the examined calves, the number of molecules of actin and myosin for 1-2 days was 20.9 ± 0.14 and $9.8 \pm 0.15\%$ of the total protein in platelets, dropping to 19.3 ± 0.04 by the end of observation and $8.9 \pm 0.14\%$ of total protein in platelets. Self-assembly of actin and myosin in the course of platelet aggregation in Dutch calves during the neonatal phase also experienced a slight downward trend.

Studies on various physiological aspects of blood have been conducted for quite some time (Shitikova, 2000). They helped to gather a large amount of information, which allows finding out various aspects of regulatory mechanisms in mammals (Korepanova et al., 2015). It becomes clear that platelet activity has a greater physiological significance in the organism of animals. At the same time, its condition in young cattle of highly productive cattle breeds is still very poorly studied. In the work performed, an attempt was made to elucidate the peculiarities of platelet activity in newborn calves, a Dutch breed.

When evaluating the time of AP development in the examined calves in response to collagen and ristomycin, it was possible to note the initial small adhesive activity of their platelets, which had a tendency to weaken during the neonatal period. It is clear that these changes were based on at least two biologically important mechanisms (Zavalishina, 2018b). First, calves have a tendency to weaken platelet aggregation, which develops in response to the appearance of collagen in plasma (Zavalishina, 2018c). The basis of this phenomenon is the development of a decrease in initially low density on the membranes of platelets in calves of collagen receptors - glycoproteins Ia-IIa and VI (Vorobyeva et al, 2018). The presence of a second mechanism to ensure low platelet adhesion in calves of the Dutch breed was indicated by a tendency for AP to weaken in response to ristomycin (Zavalishina, 2018e). This biological mechanism was associated with the development of a weak decrease in the concentration of von Willebrand factor in the blood of calves during the neonatal phase and the inactive involvement of receptors to it (GPIb) on the platelet surface in the adhesive process (Zavalishina, 2018f).

In the work it was found out that for newborn Dutch calves a tendency to inhibition of initially inactive platelet aggregation is characteristic. There is reason to believe that these changes are designed to improve blood circulation processes in microvessels, (Zavalishina, 2018g). The initial low sensitivity of platelets to the stimulators of the aggregation process, which at the same time has a tendency to weaken, was also manifested by inhibition of interaction with the platelets of strong

aggregation inducers - collagen and thrombin (Usha et al., 2019). Obviously, these changes were based on the weakening of the activity of the phospholipase C and the whole phosphoinositol mechanism, which were combined with a small degree of phosphorylation of the proteins of the actino-myosin complex (Zavalishina, 2018i). The optimally low production of inositol triphosphate in their platelets, apparently, provided a small degree of Ca^{2+} release from its depot and contributed to a decrease in the intensity of actomyosin self-assembly and a decrease in its reduction.

Considered weak agonists of the platelet aggregation process, ADP and adrenaline provided a low degree of severity in calves of the Dutch breed at birth, which tended to decrease during neonatality (Zavalishina, 2018k). These changes were obviously based on a low density of receptors for them on the platelet surface, a small degree of expression of fibrinogen receptors (GPIIb-IIIa), and low activation of phospholipase A2 during platelet aggregation. This provided a functional minimum of the yield of arachidonic acid from membrane phospholipids, which limited the synthesis of thromboxane A2 (Zavalishina, 2018l). At the same time, the activity of platelet cyclooxygenase and thromboxane synthetase was low in calves of the Dutch breed, which also restrained the synthesis of thromboxane A2 aggregate. This circumstance was proved by the results of carried out transfer tests, which made it possible to detect low activity of platelet cyclooxygenase and thromboxane synthetase in the blood plates of calves. A low AP level in newborn Dutch-born calves with all inductors was also ensured by their basal actin formation and myosin formation during platelet aggregation and unexpressed secretion of ATP and ADP platelets from granules (Shitikova, 2000).

Detection in the blood of Dutch calves of a low level of active forms of platelets proved in them a low sensitivity of platelets to any inducers of aggregation. The basal level of intravascular platelet activity found in them also proved the poor availability of collagen in their vascular wall due to the high endothelial integrity. This circumstance was ensured by the presence in the blood of the observed calves of a small number of active species of platelets and their aggregates. Indirectly, this confirmed the low sensitivity of Dutch-born calves' platelets to the aggregation inducers (ADP, thrombin, adrenaline) that are constantly present in their blood (Zavalishina, 2018m).

A decrease in the initially low aggregation ability of platelets in the observed calves caused a decrease in the level of their active forms and their aggregates of any size circulating in the blood. The found changes can be considered as an important mechanism for ensuring low activity of platelet hemostasis, optimum hemocirculation of capillaries and maintaining platelet vascular relationships at a physiologically beneficial level. The revealed small intravascular platelet activity in the newborn calves of the Dutch breed indicated a low activity of the adhesive and aggregation properties of

the platelets exhibited *in vivo*. Taking into account the data given in the literature (Kulikov et al., 2019), it can be assumed that the platelets of newborn calves of the Dutch breed have a very pronounced ability to disaggregate. This is due to the high sensitivity of their receptors to antiaggregant substances of vascular origin.

CONCLUSION

Newborn calves of the Dutch breed have a high degree of functional perfection of platelets. It largely provides physiologically favorable conditions for microcirculation and tissue metabolism. This is possible as a result of the low activity of their platelet mechanisms that ensure the flow of adhesion, aggregation and secretion. Small intravascular platelet activity in newborn calves of the Dutch breed contributes significantly to the formation of the optimum of their overall viability, which is required for their rapid growth and development.

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