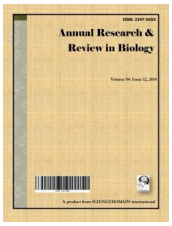

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Platelet Parameters of Holstein Newborn Calves

N. V. Kutafina[1*]

[1]All-Russian Research Institute of Physiology, Biochemistry and Nutrition of Animals, Institute of Village, Borovsk, Russia.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The activity of cattle's platelets is mostly connected with the level of their metabolic processes. It is especially important at the beginning of ontogenesis and can differ in different breeds. The aim of the study is to estimate platelets' activity of healthy Holstein calves during the newborn phase. The study used 35 Holstein calves which were received from healthy cows with the normal course of in-calf state. The calves were observed and examined on the 1^{st}-2^{nd}, 3^{rd}-4^{th}, 5^{th}-6^{th}, 7^{th}-8^{th} and 9^{th}-10^{th} days of life. We used biochemical, hematological and statistical methods of investigation. During the newborn phase the calves had a trend to weakening of platelets' aggregation in response to all the applied inductors. Discocytes' content in blood of Holstein calves during the newborn phase had an upward trend. The sum of platelets' active forms in their case had an inclination for lowering on 7.6%. The number of freely moving little and large platelets' aggregates in blood also had a downward trend during the first 10 days of ontogenesis. It was evidently provided by a trend to weakening of thromboxane synthesis in platelets of calves and decreasing of adenosine phosphates' content in them at inclination for weakening of their secretion. The quantity of actin and myosin in platelets of observed calves on the 1^{st}-2^{nd} days of life was little and had an inclination for lowering in the course of the newborn phase. Having conducted the given study, we can consider that Holstein newborn calves are characterized by high functional perfection of platelet hemostasis. Low intravascular activity of platelets in Holstein newborn calves provides optimum of perfusion and metabolism in all the internals which are necessary for fast growth and development of animals.

**Corresponding author: E-mail: kutafina.nv@mail.ru;*

Keywords: Calves; newborn phase; Holstein; platelets; aggregation; secretion.

1. INTRODUCTION

The activity of hemostasis system mostly determines the success of blood circulation along vessels [1,2]. Platelets are rather important in this aspect. Their level of activity mostly determines the state of microcirculation [3,4] in different biological objects [5,6]. It was noted that functional characteristics of platelets could be changed in the process of growth and development [7], in the process of aging [8], at development of different dysfunctions [9,10], appearance of somatic pathology in a body [11,12], emergency of vascular abnormalities [13,14] and carrying out of different curative measures [15-17]. At the same time, various aspects of cattle's platelet hemostasis have been studied rather poorly. There are just several works which are devoted to platelets' activity of these productive animals at some stages of their ontogenesis [18]. It doesn't allow making a coherent picture of it and dictates the necessity of further investigations. Besides, there is some ground to consider that some very important factors are mostly connected with the level of platelets' activity in cattle. They are as follows: the intensity of capillary bloodstream, speed of body structures' development and level of their functional activity. They are based on the realization of hereditary information in the course of ontogenesis [19]. There are a lot of genetic and physiological differences between cattle breeds. That's why, it's rather interesting to estimate platelets' activity of Holstein calves during the newborn phase. This breed is famous for its milk productivity.

The aim is to estimate platelets' activity of healthy Holstein calves during the newborn phase.

2. MATERIALS AND METHODS

The 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 in March 18th, 1986, and confirmed in Strasbourg in June 15th, 2006) and approved by the local Ethics Committee of All-Russian Scientific Research Institute of Physiology, Biochemistry and Animals' Feeding (record №11, dated December 4th, 2015).

The study was conducted on "Kolos" farm in 2016. The farm was situated in Kursk region (Russia). The study used 35 Holstein calves which were received from healthy cows with the normal course of in-calf state. The calves were observed and examined 5 times during the newborn phase: on the 1st-2nd, 3rd-4th, 5th-6th, 7th-8th and 9th-10th days of life.

Indirect estimation of the level of thromboxane synthesis in platelets and indirect determination of cyclo-oxygenase and thromboxane-synthetase activity in them were conducted with the help of three transfer tests in which we determined platelets' aggregation on photo-electro-colorimeter [20]. We determined the quantity of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) content in platelets, the level of their secretion in response to collagen. We also determined the level of actin and myosin in cytoskeleton composition of inactive platelets and in platelets in the course of their aggregation under the impact of ADP [20] with recalculation of the obtained results in accordance with the level of total proteins in platelets [21].

Evidence of platelets' aggregation (AP) was registered by visual micro-method [22] with the application of ADP (0.5×10^{-4} M), collagen (dilution 1:2 of the basic suspension), thrombin (0.125 un/ml), adrenaline (5.0×10^{-6} M) and ristomicin (0.8 mg/ml) as inductors in plasma with standardized number of platelets till 200×10^{9} tr. Intravascular platelets' activity (IAP) was determined with the help of phase-contrast microscopy [23].

The results were processed by Student's criterion (t). Statistical processing of received information was made with the help of a program package "Statistics for Windows v. 6.0", "Microsoft Excel". Differences in data were considered to be reliable in case of $p<0.05$.

3. RESULTS AND DISCUSSION

Holstein calves during the newborn phase had a downward trend of initially low platelets' activity. So, on the 1st-2nd days of life the examined animals got AP in response to collagen for 37.6±0.11s, by the 9th-10th days of life there was some inhibition till 38.2±0.12s. The same trend to AP lowering was found in response to ADP and ristomicin till 47.6±0.08s and 55.8±0.17s, respectively. There was also found an inclination

for AP inhibition with thrombin (till 59.6±0.25s) and with adrenaline (till 108.2±0.25s).

The content of discocytes in blood of the examined calves during the newborn phase had an upward trend. At the same time, in the course of our research the sum of platelets' active forms had an inclination for lowering on 7.6%. The number of freely circulating little and large platelets' aggregates in blood also had a downward trend for the first 10 days of ontogenesis.

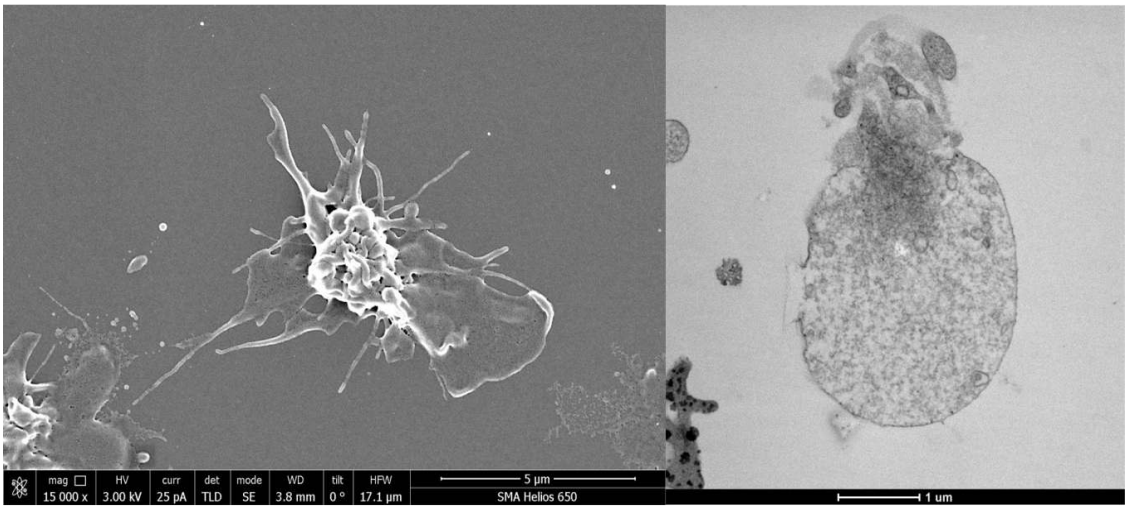

Fig. 1. Ultrastructure of a platelet
http://www.msu.ru/upload/iblock/93f/panteleev_1.jpg

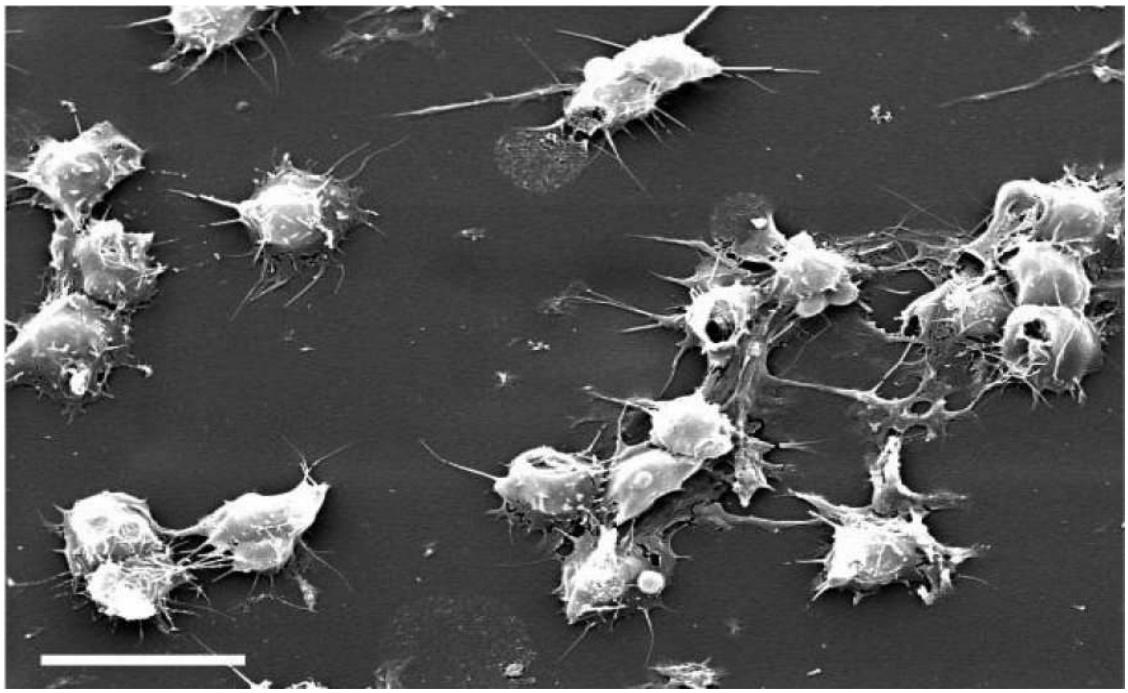

Fig. 2. Morphological consequences of actin and myosin contraction in platelets during their activation
https://images.sciencedaily.com/2011/10/111018211341_1_900x600.jpg

Table 1. Platelet indices of Holstein newborn calves

Indicators under consideration	Calves of Holstein breed, n=35, M±m				
	1-2 day	3-4 day	5-6 day	7-8 day	9-10 day
value of platelets' aggregation reduction in collagen-aspirin test, %	75.6±0.10	75.3±0.14 p<95%	74.4±0.09 p<95%	73.6±0.12 p<95%	73.1±0.08 p<95%
the level of platelets' aggregation reduction in collagen-imidazole test, %	36.2±0.06	36.0±0.09 p<95%	35.5±0.08 p<95%	35.0±0.10 p<95%	34.4±0.09 p<95%
platelets' aggregation lowering in a simple transfer test, %	26.3±0.08	25.9±0.10 p<95%	25.6±0.07 p<95%	25.4±0.09 p<95%	25.1±0.11 p<95%
Content of ATP in platelets prior to secretion, mkmol /10^9 platelets	5.31±0.014	5.30±0.012 p<95%	5.28±0.009 p<95%	5.26±0.014 p<95%	5.24±0.006 p<95%
The maintenance of ADP in platelets prior to secretion, mkmol /10^9 platelets	3.17±0.007	3.16±0.008 p<95%	3.15±0.010 p<95%	3.14±0.006 p<95%	3.12±0.008 p<95%
Secretion level ATP,%	25.2±0.14	25.2±0.10 p<95%	25.0±0.15 p<95%	24.7±0.12 p<95%	24.4±0.11 p<95%
Secretion level ADP,%	32.5±0.18	32.4±0.15 p<95%	32.4±0.19 p<95%	32.2±0.21 p<95%	31.8±0.17 p<95%
The number of actin in inactive platelets,% of total protein in platelets	20.6±0.12	20.4±0.10 p<95%	20.4±0.07 p<95%	20.2±0.08 p<95%	20.0±0.11 p<95%
The number of actin in platelets with ADP-aggregation,% of total protein in platelets	32.1±0.12	32.0±0.13 p<95%	32.0±0.16 p<95%	31.9±0.11 p<95%	31.8±0.10 p<95%
Number of myosin in inactive platelets,% of total protein in platelets	9.8±0.16	9.6±0.18 p<95%	9.6±0.14 p<95%	9.5±0.18 p<95%	9.0±0.15 p<95%
Number of myosin in platelets with ADP-aggregation,% of total protein in platelets	21.9±0.15	22.0±0.13 p<95%	21.7±0.10 p<95%	21.6±0.17 p<95%	21.5±0.19 p<95%
Aggregation of platelets with ADF,s	46.8±0.12	46.8±0.14 p<95%	47.0±0.10 p<95%	47.5±0.13 p<95%	47.6±0.08 p<95%
Aggregation of platelets with collagen, s	37.6±0.11	37.6±0.09 p<95%	37.8±0.12 p<95%	38.0±0.07 p<95%	38.2±0.12 p<95%
Aggregation of platelets with thrombin, s	58.9±0.19	59.2±0.22 p<95%	59.4±0.20 p<95%	59.6±0.18 p<95%	59.6±0.25 p<95%
Aggregation of platelets with rhistomicin, s	55.0±0.19	55.3±0.17 p<95%	55.2±0.21 p<95%	55.6±0.26 p<95%	55.8±0.17 p<95%
Aggregation of platelets with adrenaline, s	107.1±0.26	107.3±0.29 p<95%	107.4±0.21 p<95%	107.6±0.32 p<95%	108.2±0.25 p<95%
Platelets-discocytes, %	84.4±0.17	84.5±0.21 p<95%	85.0±0.24 p<95%	85.4±0.19 p<95%	85.5±0.26 p<95%
Sum of platelets active forms, %	15.6±0.16	15.5±0.20 p<95%	15.0±0.24 p<95%	14.6±0.17 p<95%	14.5±0.19 p<95%
Number of little aggregates (in 100 free platelets)	2.7±0.08	2.7±0.07 p<95%	2.6±0.09 p<95%	2.5±0.05 p<95%	2.5±0.06 p<95%
Number of medium and large aggregates (in 100 free platelets)	0,08±0,022	0.07±0.026 p<95%	0.07±0.017 p<95%	0.06±0.020 p<95%	0.06±0.025 p<95%

Note: reliability of indices' dynamics in relation to 1-2-days age wasn't found

As one of the mechanisms which caused a trend to AP weakening in Holstein calves during the newborn phase, we could consider the found trend to weakening of thromboxane synthesis in their platelets. It was indirectly judged by AP lowering in a simple transfer test (on the 9^{th}-10^{th} days of life – 25.1±0.11%). It was provided in experimental animals by a trend to activity weakening of the synthesis of both enzymes – cyclo-oxygenase and thromboxane-synthetase – in their platelets. So, the value of AP reduction in collagen-aspirin test, which indirectly characterized the activity of cyclo-oxygenase in platelets, reached 73.1±0.08% by the end of the research. At the same time, the level of AP reduction in collagen-imidazole test, which gave possibility to estimate indirectly the activity of thromboxane-synthetase in platelets, also lowered in experimental calves during the research and reached 34.4±0.09% by the 9^{th}-10^{th} days of calves' life.

Initially low ATP and ADP content in animals' platelets had an inclination for lowering and reached 5.24±0.006 and 3.12±0.008 mkmol/10^9 of platelets by the end of the newborn phase. At the same time, the levels of their secretion out of platelets during the research had a trend to weakening on 3.3% and 2.2% and reached 24.4±0.11 and 31.8±0.17% by the end of the research.

The quantity of actin and myosin in inactive platelets of experimental calves was equal to 20.6±0.12 and 9.8±0.16% of common protein in a platelet on the 1^{st}-2^{nd} days of life. By the end of the newborn phase it was equal to 20.0±0.11 and 9.0±0.15% of common protein in a platelet. Additional formation of actin and myosin against the background of platelets' aggregation in Holstein calves also had a downward trend in the course of the whole newborn phase.

Nowadays hematological researches in biology and medicine are conducted rather actively as they can help in further exposure of many aspects of regulatory mechanisms in a mammal's body [24,25]. Notwithstanding the great physiological signification of platelet activity in young cattle of highly productive breeds, it has been studied rather poorly. We were the first to find peculiarities of platelets' activity in Holstein newborn calves in our study.

While conducting AP estimation with collagen and ristomicin in calves we managed to find initially low adhesion ability of their platelets. This ability still had an inclination for lowering during the newborn phase. It was evidently provided by, at least, two mechanisms [26]. The first mechanism was found according to the trend to inhibition of platelets' aggregation in response to collagen. It was connected with the development of an inclination for lowering of initially low quantity of receptors to collagen - glycoproteids Ia – IIa and VI on platelets' membranes of calves for the first 10 days of their life. The presence of the second mechanism of provision of platelets' low adhesion ability was found in Holstein calves with the help of the trend to AP lowering with ristomicin. Given mechanism was connected with an inclination for lowering of initially low concentration of von Willebrand Factor in their blood during the newborn phase and, on behalf of this, weak involvement into the process of receptors' (GPI b) adhesion to it on platelets' membranes.

In the course of our study we found out that development of a trend to deceleration of initially low platelets' aggregation was attributable for newborn Holstein calves. It could rather positively influence the process of microcirculation in tissues. In conditions of initially low sensitivity of platelets to inductors of aggregation which had a downward trend, the connection of strong aggregation inductors – collagen and thrombin – with their own receptors was inhibited. It promoted activity repression of phospholipase C and phosphoinositol way at unexpressed phosphorylation of contractile system's proteins. Inactive formation of inositol triphosphate in their platelets provided (according to literature data) repression of Ca^{2+} yield out of its repository. To some extent, it was the cause of weakening of their self-assembly process and reduction of actomyosin [27].

Weak inductors of platelets' aggregation (ADP and adrenaline) also caused low aggregation in Holstein calves. This aggregation had a trend to weakening in the course of the research. It was evidently provided by little number of receptors to them on platelet membranes, physiologically minimum expression of fibrinogenic receptors (GPIIb-IIIa) and low activity stimulation of phospholipase A_2 during the process of aggregation. The last mechanism provided the yield of arachidonic acid limited quantity out of membrane phospholipids and promoted inhibition of thromboxane A_2 synthesis [28]. Besides, Holstein calves had low functional ability of cyclo-oxygenase and thromboxane-synthetase of platelets which also provided generation of

thromboxane A_2 physiologically minimum quantity. It was confirmed by the results of conducted transfer tests. In platelets of Holstein calves we found low activity of both enzymes of arachidonic acid transformation into thromboxane – cyclo-oxygenase and thromboxane-synthetase. As one of important mechanisms of low AP provision in newborn Holstein calves we should also consider found in them low actin- and myosin-formation in response to the impact of aggregation inductors and moderate platelet secretion of ATP and ADP.

For estimation of initial stages of newborn Holstein calves' peculiarities of platelets' activation in vivo we used IPA method of estimation with the help of phase-contrast microscopy. Found in the course of our research little quantity of platelets' active forms in experimental animals' blood confirmed their lowered sensitivity to aggregation inductors. Low IPA level also pointed at the presence of low availability of vascular wall collagen because of high undamaged state of endothelium. It was the result of little quantity of freely circulating platelets' active forms and their aggregates in animals' blood. It also indirectly pointed at weak contact of these fibers with platelets and low level of other aggregation inductors (ADP, thrombin, and adrenaline) in blood of Holstein calves. Found downward trend of low platelets' aggregative ability of experimental animals caused inclination for content lowering of their active forms and their dynamic aggregates of different sizes. Given circumstance should be considered as an important mechanism of repression of platelet hemostasis activity, minimization of blockade risk of functionally significant capillaries' quantity by platelets' aggregates and maintenance of platelet-vascular interactions on the optimum level [29]. Found low intravascular platelets' activity in newborn Holstein calves certified not only low activity of platelets' adhesive and aggregative abilities in vivo but also allowed supposing (basing on literature data [30,31]) the presence of high, physiologically rather favorable disaggregative ability in them. It was evidently connected with high sensitivity of their receptors to vascular antiaggregates [32].

4. CONCLUSION

Newborn Holstein calves are characterized by high functional perfection of platelet hemostasis which provides physiologically rather favorable conditions for microcirculation. It is provided by low activity of mechanisms which realize platelets' adhesion, aggregation and secretion. Low intravascular platelets' activity of newborn Holstein calves provides the optimum of perfusion and metabolism in all the internals which are necessary for quick growth and development of animals.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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