Cell-mediated transfer of immune protection factors to newborn calves

Lyudmila Proskurina[1], *Elena* Enns[2], *Margarita* Simakova[1], *Natalia* Repsh[3,*], and *Dmitriy* Zamaratskiy[4]

[1]Primorskaya State Academy of Agriculture, pr. Blyukhera, 44, Ussuriysk, Primorsky Territory, 692510, Russia
[2]Innovative University of Eurasia, ul. Lomova, 45, Pavlodar, 140000, Kazakhstan
[3]Far Eastern Federal University, ul. Sukhanova, 8, Vladivostok, Primorsky Territory, 690090, Russia
[4]Ussurian Veterinary Station of Animal Diseases Control, ul. Kolkhoznaya, 81, building A, Novonikolsk, Primorsky Territory, 692537, Russia

Abstract. Immunocompetent cells in the blood, colostrum (milk) of cows and in the blood of newborn calves obtained from these animals give grounds to assert that newborn calves acquire cellular immune protection due to the intake of colostrum. With the colostrum of cows, not only nonspecific, but also specific immunocompetent cells enter the newborn's body. The creation of colostral immunity is determined by the presence of a sufficient number of protective factors in colostrum and increased permeability of the intestinal histohematogenous barrier, which is capable of passing large immunoglobulin bodies and lymphocytes unchanged in the first 24-36 hours of life. A large number of specific immunocompetent cells found in colostrum is directly related to their increased content in the blood of these animals. The strength of the immune response depends on the preservation of immunological memory cells in the blood of revaccinated animals, some of which are represented in the pool of brucellin-reactive T-lymphocytes.

1 Introduction

The viability of the newborn to a certain extent depends on the level of immune protection factors acquired with colostrum in the first days of life. One of the requirements for rearing young animals is that the growth matches the physiological development of the body. For animals of dairy breeds, an earlier development is desirable for the earliest achievement of the period of their practical use [1].

J.M. Rodriguez and I.V. Ivanova's research confirmed the assumption of many authors that living cells of colostrum are able to pass through the epithelial barrier of the intestine into the system of the newborn [2, 3].

Information about the cellular components of colostrum and cow milk involved in the protection of newborn calves is insufficient and contradictory. In the literature, there is no

* Corresponding author: repsh_78@mail.ru

data on the study of specific cellular factors of the secretion of the mammary gland, which is especially important for understanding the ability of the mother's body to transfer immune components to the newborn that protect it from a certain infection.

It is considered recognized that animals in the first days of life are characterized by immunological immaturity associated with poor development of lymphoid tissue and the absence of immunocompetent cells. The maternal organism compensates for this immaturity of the newborn by transferring ready-made antibodies to it, for a short time protecting the offspring from a number of pathogens [4, 5].

We made an attempt to clarify the mechanism of transmission of immune factors (including specific immunocompetent cells) from cows vaccinated against brucellosis to newborn calves.

2 Materials and methods

The work was carried out in the laboratory of the Department of Human and Animal Morphology of the Semey State University and the private enterprise “Prirechnoye”.

The object of the study was the blood, colostrum (milk) of Kazakh white-headed cows, revaccinated against brucellosis before mating with a vaccine from the Brucella abortus 82 strain, and the blood of calves obtained from these cows.

The method for detecting T-lymphocytes, their subpopulations and brucellin-reactive T-cells was based on the generally recognized method of spontaneous rosette formation (RF) with sheep erythrocytes [6], modified [7] for cattle. To determine subpopulations of lymphocytes, the theophylline method was used — the sensitivity of immunocompetent cells [8].

Brucellin-reactive T-cell clone was determined by the method of P.J. Felsburg, R. Edelman [9], and brucellin-reactive clone of T-helpers - by the method proposed by L.I. Proskurina [10].

The number of B-lymphocytes was determined by the reaction of complementary rosette formation (EAC-RF) according to N. Mendes. Bovine erythrocytes loaded with hemolysin and complement were used as an indicator system [11].

Brucellin-reactive population and a subpopulation of B-lymphocytes, were detected similarly to T-cells [12].

3 Research results

Analysis of the research results of nonspecific cellular components of the blood of cows showed (table 1) that the total number of leukocytes and lymphocytes significantly ($P<0.01$ - 0.01) decreases on the 7th day after calving from 5.4 to 3.3 and from 3.5 to 2.2 thousand in μl.

The relative and absolute number of B-cells also significantly ($P<0.05$ - 0.01) decreases during this period. No significant changes were observed on the part of the populations and subpopulations of T-lymphocytes.

The number and percentage of brucellin-reactive - B-, T- and Tn-cells in the blood of cows change identically (table 2). So, the number of these cells increases on the 2nd day after calving, and on the 4th day it reaches a maximum. Moreover, the percentage and number of brucellin - reactive B-lymphocytes and brucellin - reactive T-helpers increase ($P<0.05$ - 0.01; $P<0.01$ - 0.05) significantly (from 14 to 47%); (from 64 to 144 in μl); (from 5.8 to 32.7%); (from 7 to 29 in μl). Thirty days after calving, the number of these cells approaches the initial level.

Brucellosis antibodies were not detected in the blood of cows throughout the experiment.

At the same time, attention is drawn to the fact that after calving, brucellin - reactive B-lymphocytes and brucellin - reactive T-helpers are detected in the blood of cows in amounts (14 and 20.3%) slightly exceeding their level in animals one month before calving.

The number of brucellin-reactive cells increases sharply on the 2nd day after calving and remains elevated for seven days.

Table 1. The content of immunocompetent cells in the blood of cows reimmunized before mating with a vaccine from strain 82 (M±m).

Research period, days	Quantity			B-cells		T-cells		T-helpers		T- suppressors	
	leukoc., th. µl	lymph., percent	lymph., th. µl	percent	abs., µl	percent	abs., µl	percent	abs., µl	percent	abs., µl
Before calving	5.1±0.2	63.2±4.0	3.2±0.3	13.2±0.7	425±50	22.0±2.9	709±71	10.8±0.69	76±11	9.0± 1.2	64±9
After calving	5.4±0.3	64.5±5.3	3.5±0.4	13± 0.84	455±55	23.5±3.9	826±79	11.5±0.84	97±15.9	9.5± 1.4	78±13
After calving on the 2nd day	4.9±0.42	66±9.5	3.2±0.4	11.5±1.7	369±54	24.5±3.4	809±196	12.7±1.1	106±31	6.5±1.9	48±11.7
4th day	4.8±0.4	62.5±6.7	2.9±0.1	11.2±1.9	325±50	30±5	883±167	11± 3.4	104±49	14±4.5	137±58
7th day	3.3±0.4	67±6.2	2.2±0.1	9±1.1	193±11	26.7±5.6	573±87	15± 3.9	92±34	5.7±1.7	35±15
14th day	4.97±0.5	74±6.4	3.6±0.5	11.5±0.56	414±46	25.5±4.5	895±110	12± 2.5	110±33	6.2±1.7	55±19
30th day	6.0±0.6	69.2±6.2	4.1±0.08	9.2±2.5	382±121	23±4.4	939±168	12.2±3.4	124±52	7±1.6	73±26

Note: n = 4 at each study period.

Based on the data obtained, we believe that the increase in the pool of brucellin-reactive cells in the blood of cows revaccinated before mating is caused by memory cells.

After calving, on the 2nd day of observation (table 3), a large number of leukocytes (11.5-12.2 thousand μl) are detected in the colostrum of cows. On the 4th day of lactation, the total number of leukocytes in colostrum decreases (P= 0.001) by half (6.3 thousand μl), and by one month their number (P<0.001) does not exceed 0.9 thousand μl, which is more than twelve times the baseline.

In the cytogram of cows' colostrum, a significant number of lymphocytes (92-92.5%) are detected immediately and on the 2nd day after calving. On the 14th day, a significant (P<0.01) decrease in the percentage of lymphocytes (74.7) is recorded, and by the end of observations, their number becomes significantly lower than the pre-experimental level. The total number of lymphocytes in colostrum begins to decrease significantly (P <0.001) on the 4th day of lactation (from 10.5 to 5.5 thousand μl). In the remaining periods of the study, the total number of lymphocytes in colostrum (milk)decreases, and on the 30th day, their number becomes (P<0.001) twelve times less (0.52 thousand μl) of the preliminary indicator.

The relative number of B-, T-cells detected in colostrum (milk) during all periods of observation did not undergo significant changes. At the same time, their absolute number begins to decrease (P<0.05-0.01) on the 4th, 7th day of lactation (1307-634 and 779-225 in μl). At the end of the experiment, their number becomes15 and 18 times lower than the initial level, respectively.

Subpopulations of T-lymphocytes in colostrum (milk) of cows are found similarly to the general clone of T-cells.

In colostrum in the first hours after calving (table 4), a significant amount of brucellin-reactive cells was detected. So, during this period, the percentage of brucellin-reactive B-lymphocytes was 58.6, brucellin-reactive T-lymphocytes - 41, - brucellin-reactive T-helpers - 56, which is significantly higher than the level of these cells detected in the blood of these animals.

Starting from the fourth day, a decrease in the pool of brucellin-reactive cells is found in the colostrum of cows, and by one month, their number and percentage becomes significantly (P<0.01-0.001) less than the preliminary indicator.

Table 2. The content of brucellin-reactive cells and antibodies in the blood of cows reimmunized before mating with a vaccine from strain 82 (M±m).

Research period, days	Brucellin-reactive						Reverse tyrosine in RA
	B-cells		T-cells		T-helpers		
	percent	abs., μl	percent	abs., μl	percent	abs., μl	
Before calving	12±0.9	51±10	9.2±1.3	65.2±9.1	5.5±4.0	4.2±1.3	0
After calving	14±1.9	64±11.2	20.3±3.2	164±11	5.8±4.3	7±5	0
After calving on the 2nd day	26.3±10.5	103±53	21.9±3.6	183±56	21±12	27±21	0
4th day	47±11	144±15	40±12	387±162	32.7±6.5	29±8.1	0
7th day	23.4±9.2	46±17.6	9.8±3.7	52±12.9	24.3±7.7	18±2.8	0
14th day	13.1±8.4	54±33	15.1±8.5	135±78	15.9±6.1	14±3.4	0
30th day	9.5±5.6	51±32	4.7±2.5	35±15	7±2.9	11±6.7	0

Note: n = 4 at each study period.

In the study of blood obtained from newborn calves, first of all, attention is drawn to the fact that newborn calves, before taking colostrum, are characterized by weak activity of cellular immunity (table 5).

In the blood cytogram, an insignificant content of lymphocytes is found, B-, T- cells and their subpopulations have a low, or even zero, rosette forming ability. On the second day after taking colostrum, the number and percentage of lymphocytes doubles (P<0.01). The population of B- and T-cells increases significantly (P<0.05-0.01) in the blood of calves, while Tn- and Ts-lymphocytes are still not detected during this period.

Table 3. The content of immunocompetent cells in colostrum (milk) of cows reimmunized before mating with a vaccine from strain 82 (M±m).

Research period, days	Quantity			B-cells		T-cells		T-helpers		T- suppressors	
	leukoc., th. µl	lymph., percent	lymph., th. µl	percent	abs., µl	percent	abs., µl	percent	abs., µl	percent	abs., µl
After calving	11.5±1.1	92±3.4	10.5±0.84	7.2±1.4	779±194	12.2±1.4	1307±208	5.5±0.84	74±18	5.5±0.56	73±19
After calving on the 2nd day	12.2±1.2	92.5±3.1	11.3±1.2	4.5±0.84	529±147	13±1.4	1487±243	4.5±0.84	69±21	6±0.84	91±27
4th day	6.3±0.28	87±3.1	5.5±0.1	5±1.4	275±74	11.5±0.84	634±42	6.7±2.2	44±17	2.7±4.4	20±8,9
7th day	3.8±0.7	84.5±4.5	3.2±0.4	7±1.1	225±48	11.5±1.1	364±67	5±1.4	19±7	5±1.9	17.5±5
14th day	2.3±0.4	74.7±4.2	1.7±0.4	7±0.56	117±18	11.7±0.84	195±33	5.5±0.84	11±2.5	5±0.84	9±1.1
30th day	0.9±0.1	54.7±5.3	0.52±0.1	8±0.56	42±10,3	16±1.9	85±24	7.7±1.4	7±1.9	6.2±1.6	6±3.4

Note: n = 4 at each study period

Table 4. Content of brucellin-reactive cells in colostrum (milk) of cows reimmunized before mating with vaccine from strain 82 (M±m).

Research period, days	Brucellin-reactive					
	B-cells		T-cells		Ti-cells	
	percent	abs., µl	percent	abs., µl	percent	abs., µl
After calving	58.6±8.9	426±43	41±2.3	531±81	56±4.7	42±12
After calving on the 2nd day	39.6±5.9	200±51	38.3±9.3	534±159	56.7±4.7	38±9.5
4th day	28.4±5.6	76±23	24±5.8	295±167	35±11.6	12±2.5
7th day	22.2±5.8	54±20	12.4±2.5	43±3.6	29±9.9	5±2.2
14th day	13.1±5.6	17±8.4	13.3±7	28±15	21.7±9.3	2±1.4
30th day	11.9±5.6	5±3.6	3.9±2.8	3±2.2	8.5±8.4	1±0.56

Note: n = 4 at each study period

Table 5. The content of immunocompetent cells in the blood of calves obtained from cows reimmunized before mating with a vaccine from strain 82 (M±m).

Research period, days	Quantity			B-cells		T-cells		T-helpers		T- suppressors	
	leukoc., th. µl	lymph., percent	lymph., th. µl	percent	abs., µl	percent	abs., µl	percent	abs., µl	percent	abs., µl
Before taking colostrum	5.0±0.6	41±8.9	2.1±0.6	0.5±0.56	17±19.6	1.5±0.84	36±29	0	0	0	0
After taking colostrum on the 2nd day	5.0±0.6	81±7.8	4.1±0.7	4±1.4	155±46	8±1.7	322±54	0	0	0	0
4th day	5.0±0.4	75±3.6	3.8±0.3	5.2±1.1	199±39	11±0.56	417±49	5±0.56	21±5.0	5.2±0.84	22±3.1
7th day	4.8±0.5	81.5±5	3.9±0.5	8±0.56	307±40	14±2.2	538±99	4.5±0.84	25±7	7.5±2.5	43±14.6
14th day	5.4±0.56	77±2.5	4.1±0.4	8.2±1.4	339±61	16.2±2.2	667±101	7.5±1.4	52±13	6±0.84	41±8.9
30th day	5.7±0.6	76.7±4.2	4.3±0.2	7.5±0.84	325±34	18±2.8	780±102	8±0.84	64±12.9	7±2.2	58±24

Note: n = 4 at each study period.

Table 6. The content of brucellin-reactive cells in the blood of calves obtained from cows reimmunized before mating with a vaccine from strain 82 (M±m).

Research period, days	Brucellin-reactive					
	B-cells		T-cells		Ti-cells	
	percent	abs., μl	percent	abs., μl	percent	abs., μl
Before taking colostrum	0	0	0	0	0	0
After taking colostrum on the 2nd day	12.5±9.3	19±12	14.6±8	50±33	0	0
4th day	48.3±7.4	95±22.5	19.4±5.8	84±31	16.2±11	3±2.2
7th day	28.1±10.5	94±4.4	44.8±8.9	218±31	47.4±9.3	11±1.9
14th day	17.9±1.4	61±14	19.1±1.8	216±17	19.7±1.6	10±3.4
30th day	11.8±4.7	37±15.7	5.4±2.8	39±16	5.9±3.5	4±2.5

Note: n = 4 at each study period

Subpopulations of T-cells begin to be detected on the 4th day after birth. Further, the number of nonspecific cells increases, and by the end of observations, (30 days) it becomes significantly (P<0.05-0.001) above the initial level.

Based on the results of the study of the brucellin-reactive clone and antibodies, it can be noted that in the blood of newborn calves before colostrum intake, there is a complete absence of antigen-reactive cells and brucellosis antibodies (table 6). A certain amount of brucellin-reactive B- and brucellin- reactive T-lymphocytes is detected on the 2nd day after taking colostrum (12.5 and 14.6%), and after four days, their number becomes (P<0.001-0.01) significant (48.3 and 19.4%), and during this period and subsequent periods, a pool of brucellin-reactive T-helpers begins to be detected. The maximum number of clone brucellin-reactive B-lymphocytes reaches (P<0.001-0.01) on the 4th day (48% and 95 in μl), -brucellin-reactive T- and brucellin-reactive T-helpers - on the seventh day (44.8% and 218 in μl; 47.4% and 11 in μl) after colostrum intake (P <0.001). With the end of the colostrum period, a decrease in the pool of brucellin-reactive cells is noted in the blood of calves, and by the end of the sucking period, a small amount of them remains.

It should be noted that brucellosis antibodies detected by RA in the blood of newborn calves were not detected before and after colostrum (milk) intake.

Thus, comparing the data obtained in the study of blood, colostrum (milk) of cows revaccinated against brucellosis before mating and blood of newborn calves obtained from these animals, it can be noted that in the blood and colostrum of cows, a clone of brucellin-reactive B-lymphocytes is detected in the first hours after calving (Fig. 1).

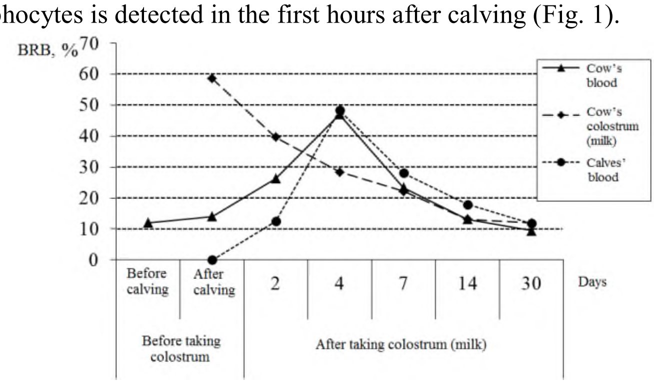

Fig. 1. The number of brucellin-reactive B-lymphocytes in the blood and colostrum (milk) of cows revaccinated against brucellosis before mating and blood of newborn calves obtained from these cows.

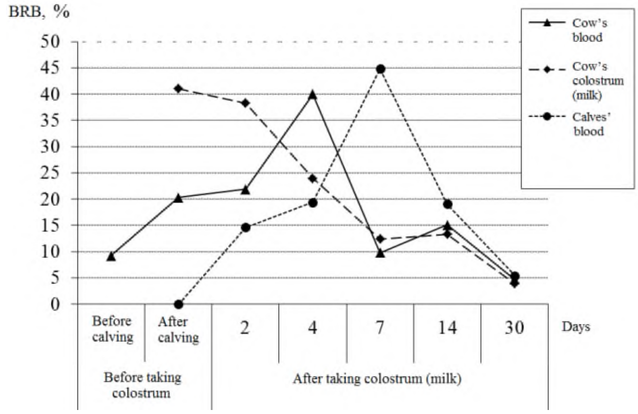

Fig. 2. The number of brucellin-reactive T-lymphocytes in the blood and colostrum (milk) of cows revaccinated against brucellosis before mating and blood of newborn calves obtained from these cows.

Moreover, in colostrum, they are found in a significant amount, compared with blood (58.6%). Two days after calving, it increases in the blood of cows (26.3%), and in the blood of calves, after taking colostrum, brucellin-reactive B-cells begin to appear (12.5%). Four days later, almost the same amount of brucellin-reactive B-lymphocytes is found in the blood of cows (47%) and newborn calves (48%). After this period, a decrease in the pool of brucellin- reactive B-cells begins in the blood and colostrum of cows and in the blood of calves. Moreover, the number of these cells varies approximately within the same limits from the fourth day.

Fig. 2 shows that in the first hours after calving, a clone of brucellin-reactive T-lymphocytes is found in the blood and colostrum of cows, and their maximum number (41%) is detected in colostrum, which is significantly (P<0.01) above the level of brucellin-reactive T-cells in the blood (20.3%).

Two days later, the number of brucellin-reactive T-lymphocytes decreases (38.3%) in the colostrum and increases (21.9%) in the blood of cows. The pool of brucellin-reactive T-cells in the blood of newborn calves begins to be detected 2 days after intake of colostrum (14.6%). The maximum of brucellin-reactive T-lymphocytes in the blood of cows was found on the 4th day after calving (40%), and in the blood of calves - on the 7th day after intake of colostrum (44.8%). After 14 days, the number of a clone of brucellin-reactive T-cells decreases in the blood, colostrum (milk) of cows and in the blood of calves. And by the end of the sucking period, the percentage of brucellin-reactive T-lymphocytes becomes insignificant.

4 Conclusion

The data obtained by us from the study of immunocompetent cells in the blood, colostrum (milk) of cows and in the blood of newborn calves obtained from these animals give reason to assert that newborn calves acquire cellular immune protection due to the intake of colostrum. With the colostrum of cows, not only nonspecific, but also specific immunocompetent cells enter the newborn's body. The creation of colostral immunity is determined by the presence of a sufficient number of protective factors in colostrum and increased permeability of the intestinal histohematogenous barrier, capable of passing large immunoglobulin bodies and lymphocytes unchanged in the first 24-36 hours of life [13, 14].

A large number of specific immunocompetent cells found in colostrum is directly related to their increased content in the blood of these animals. In our opinion, there is the circumstance that if the udder tissue is involved in antibody production [15], then it can be assumed that specific cellular factors can also originate from the udder, or antigen-reactive cells coming from the blood accumulate in colostrum.

J. Luca Lo Verso, J. Matte, J. Lapointe, G. Talbot, N. Bissonnette, M. Blais, F. Guay, M. Lessard [16] indicate that in cow colostrum after vaccination, the level of antibodies is also in several times higher than in their blood.

Our data also coincide with the results of S.N. Langel, W.A. Wark, S.N. Garst, R.E. James, M.L. McGilliard, C.S. Petersson-Wolfe, I. Kanevsky-Mullarky [17], who studied nonspecific humoral and cellular defense factors in order to establish the resistance of newborns to certain non-communicable diseases.

Apparently, it should be recognized that the strength of the immune response in brucellosis depends on the retention of immunological memory cells in the blood of revaccinated animals, some of which are presented in the pool of antigen-brucellin-reactive T- and B-lymphocytes.

References

1. G.G. Koltun, S.V. Terebova, V.V. Podvalova, I.I. Shulepova, Realization of the Genetic Potential of Imported Holstein Cattle in Agricultural Enterprises of Primorsky Krai of Russia, *Systematic Reviews in Pharmacy*, **11**, 544-549 (2020)
2. J. M. Rodriguez Microbiota of human milk, *First year of life*,**4**, 36-40. (2018)
3. I.V. Ivanova The role of breast milk in the immunological protection of a child and the formation of his immune system, *Health of Chuvashia*, **4**, 46-52. 2015
4. P. Jawor, D. Król, J. F. Mee, Z. Sołtysiak, Infection exposure, detection and causes of death in perinatal mortalities in Polish dairy herds, *Theriogenology*, 103, 130-136, 2017
5. M. Katie, B. Amy, C. Durham Chapter 13 - Bone Marrow, Blood Cells, and the Lymphoid/Lymphatic System, *Pathologic Basis of Veterinary Disease,* 724-804 (2017)
6. O.B. Zaalberg A simple metod for detecting antibody forming cells, *Nature*, **202**, 12-31 (1964)
7. V.M. Surovas On the application of the E-rosette method in the detection of rosette-forming lymphocytes in the blood and lymphoid organs of healthy and leukemic cattle // *In the book: Theoretical and practical questions of veterinary medicine*, 98-100 (1978)
8. N.K. Dambe, S. Gupta Autologos mixed lymphocyte reaction in man, *Scand. J. Immunol*, **15**, 493-499 (1982)
9. P.J. Felsburg, R. Edelman The active E-rosette test; a sensitive in vitro correlate for human delayed – type hypersensitivity, *J. Immunology*, **118**,1,62-66 (1977)
10. L.I. Proskurina Method for determination of brucellin-reactive clone of T-helpers. - *Provisional patent of the Republic of Kazakhstan*, **6720**, 10. 6 (1968)
11. A.N. Chardeev Assessment of various links of the immune system in the clinic: abstract of thesis, *Post-doctoral degree in medicine*, 40 (1981)
12. L.I. Proskurina Method for determination of brucellin-reactive B-lymphocytes. - *Provisional patent of the Republic of Kazakhstan*, **6719**, 10, (1968)

13. Yu.N. Fedorov, V.I. Klyukina, O.A. Bogomolova, Romanenko M.N. Colostrum and passive immunity in newborn calves: an overview, *Russian veterinary journal*,**6**, 20-24 (2018)
14. P. Jawor, D. Król, Infection exposure, detection and causes of death in perinatal mortalities in Polish dairy herds, *Theriogenology*, **103**,130-136 (2017)
15. D. Bucafusco, R. Pereyra Florencia, C. Mansilla Immune cells transferred by colostrum do not influence the immune responses to foot-and-mouth disease primary vaccination, *Journal of Dairy Science*, 102, 8376-8384 (2019)
16. J. Luca Lo Verso , J. Matte, J. Lapointe Impact of birth weight and neonatal nutritional interventions with micronutrients and bovine colostrum on the development of piglet immune response during the peri-weaning period, *Veterinary Immunology and Immunopathology*, **226**, 110-122 (2020)
17. S.N. Langel, W.A. Wark, S.N. Garst, Petersson-WolfeI C.S., Kanevsky-Mullarky A. Effect of feeding whole compared with cell-free colostrum on calf immune status: The neonatal period, *Journal of Dairy Science*, **98**, 3729-3740 (2015)