Медицинская иммунология
2021, Т. 23, № 4,
стр. 853-858

Краткие сообщения
Short communications

Medical Immunology (Russia)/
Meditsinskaya Immunologiya
2021, Vol. 23, № 4, pp. 853-858

РОЛЬ КЛЕТОЧНОГО ИММУНИТЕТА В РАННИЙ ПОСЛЕРОДОВЫЙ ПЕРИОД

Панова Н.А.[1], Скопичев В.Г.[2]

[1] ФГБОУ ВО «Санкт-Петербургский государственный университет ветеринарной медицины», Санкт-Петербург, Россия
[2] ФГБУ «Национальный медицинский исследовательский центр имени В.А. Алмазова» Министерства здравоохранения РФ, Санкт-Петербург, Россия

Резюме. Функционирование секреторных органов тесно связано с деятельностью иммунной системы. Как хорошо известно, это участие проявляется в том, что на определенных стадиях активности, мигрирующие в орган лимфоидные клетки, могут включаться в процесс регуляции секретообразования. Кроме того, продукты деятельности иммунной системы и даже ее клеточные элементы могут становиться составляющими ряда секретов. Молозиво и молоко содержит большое количество клеток широкого спектра (до 1/3 объема), из них численность лимфоцитов составляет до 16% от лейкоцитов. Лимфоциты в иммунологически активной форме, попадая с молозивом в организм новорожденного, активизируют систему клеточного иммунитета. Некоторую роль в этом процессе играет транспорт медиаторов лимфокининов. Микрофаги, Т- и В-лимфоциты, проникая по межклеточным пространствам в лимфоидный слой кишечника передают иммунорецепторы пролимфоцитам новорожденного, «вооружая» их активностью к распознаванию генетически чужеродного. Лимфоциты, содержащиеся в молозиве, являются клетками иммунной системы, обеспечивающими клеточный и гуморальный иммунитет. Они представлены в большей степени Т-клетками, В-клетками и клетками киллерами. Т-клетки молока вырабатывают весь спектр иммунорегуляторных белков, таких как интерферон, фактор некроза опухоли-альфа. Эти клетки являются клетками иммунной памяти. Новорожденные, получившие первую порцию молозива не позже, чем через час после рождения, отличаются повышенной численностью лейкоцитов, более выраженными фагоцитозом, что свидетельствует о стимуляции гемо- и лимфоцитоза. При проведении просвечивающей и сканирующей электронной микроскопии в эпителиальном слое кишечника обнаружены клеточные элементы, которые попали туда из просвета кишечника. На микросрезах видно, как клетки лимфоидной природы, раздвигая структуры эпителиального пласта, минуют естественные барьеры и сохраняют при этом свою физиологическую полноценность. Возможность проникновения иммунокомпетентных клеток молозива матери в кровоток детенышей доказывается при использовании естественной метки клеток самки – полового хроматина. Естественно, что меченные по половому хроматину клетки искали у новорожденных мужского пола. Обнаружение молозивных клеток в кишечной стенке и кровеносном русле детеныша примерно составляет 25% в крови, 1% в лимфе и около 70% в кишечнике. Несомненно, что лейкоциты молозива имеют исключительное значение в создании иммунитета у новорожденных животных.

Ключевые слова: лимфоциты, молоко, молозиво, иммунологическая память, Х-хромосома, половой хроматин

Адрес для переписки:
Панова Наталия Александровна
ФГБОУ ВО «Санкт-Петербургский государственный университет ветеринарной медицины»
196084, Россия, Санкт-Петербург, ул. Черниговская, 5.
Тел.: 8 (921) 758-47-47.
E-mail: panova_na@mail.ru

Address for correspondence:
Panova Natalia A.
St. Petersburg State University of Veterinary Medicine
196084, Russian Federation, St. Petersburg,
Chernigovskaya str., 5.
Phone: 7 (921) 758-47-47.
E-mail: panova_na@mail.ru

Образец цитирования:
Н.А. Панова, В.Г. Скопичев «Роль клеточного иммунитета в ранний послеродовый период» // Медицинская иммунология, 2021. Т. 23, № 4. С. 853-858.
doi: 10.15789/1563-0625-ARF-2275

For citation:
N.A. Panova, V.G. Skopichev "A role for cellular immunity in early postpartum period", Medical Immunology (Russia)/ Meditsinskaya Immunologiya, 2021, Vol. 23, no. 4, pp. 853-858.
doi: 10.15789/1563-0625-ARF-2275
DOI: 10.15789/1563-0625-ARF-2275

A ROLE FOR CELLULAR IMMUNITY IN EARLY POSTPARTUM PERIOD

Panova N.A.[a], Skopichev V.G.[b]

[a] *St. Petersburg State University of Veterinary Medicine, St. Petersburg, Russian Federation*
[b] *V. Almazov National Medical Research Centre, St. Petersburg, Russian Federation*

Abstract. The functioning of the secretory organs is closely related to the activity of the immune system. As is well known, this participation is manifested in the fact that at certain stages of activity, the lymphoid cells migrating to the organ can be involved in the regulation of secretion. In addition, the products of the immune system and even its cellular elements can become components of a number of secrets. Colostrum and milk contain a large number of cells of a wide spectrum (up to 1/3 of the volume), of which the number of lymphocytes is up to 16% of leukocytes. Lymphocytes, in an immunologically active form, entering the newborn's body with colostrum, activate the cellular immunity system. The transport of lymphokinin mediators plays a certain role in this process. Microphages, T- and B-lymphocytes, penetrating through the intercellular spaces into the lymphoid layer of the intestine, transmit immunoreceptors to the prolymphocytes of the newborn, "armed" with their activity to recognize genetically foreign ones. The lymphocytes contained in colostrum are the cells of the immune system that provide cellular and humoral immunity. They are mainly represented by T-cells, B-cells and killer cells. Milk T-cells produce a full spectrum of immune regulatory proteins such as interferon, tumor necrosis factor alpha. These cells are the cells of the immune memory. Newborns who received the first portion of colostrum no later than an hour after birth are characterized by an increased number of leukocytes, more pronounced phagocytosis, which indicates the stimulation of hemo- and lymphocytosis. When carrying out transmission and scanning electron microscopy in the epithelial layer of the intestine, cellular elements were found that got there from the intestinal lumen. Microsections show how cells of a lymphoid nature, pushing apart the structures of the epithelial layer, bypass natural barriers and, at the same time, retain their physiological usefulness. The possibility of penetration of immunocompetent cells of the mother's colostrum into the bloodstream of the young is proved using the natural label of the female's cells – sex chromatin. Naturally, sex chromatin-labeled cells were sought in male newborns. The detection of colostrum cells in the intestinal wall and bloodstream of the young is approximately 25% in the blood, 1% in the lymph, and about 70% in the intestine. There is no doubt that the leukocytes of colostrum are of exceptional importance in creating immunity in newborn animals.

Keywords: lymphocytes, milk, colostrum, immunological memory, X chromosome, sex chromatin

Introduction

Functioning of the body secretory organs is closely related to activity of immune system well recognized to be exerted in involvement of migratory lymphoid cells in controlling secretion. Moreover, immune system cues and even its cellular components may become a part of certain secretions. For instance, colostrum contains substantial amount of immunoglobulins ensuring passive neonatal immunization. Furthermore, breast milk continuously contains leukocytes (neutrophils) and lymphocytes, which amount may markedly rise upon physiological organ-wide reactions. Of note is also to describe a leukocyte inflitration into reproductive organ tissues occurring during certain alterations of hormone status. It is known that in the chain of reactions a final effect against intruding foreign protein (antigen) may not be accomplished by a single cell type, but requires sequential or simultaneous interaction between diverse cells. This phenomenon formed the basis for describing a three-cell paradigm of immunogenesis. Quantitative and qualitative characteristics of immune cells upon a cooperative response are exhibited during immune reaction. In connection with this, it is worth noting to analyze structural modules underlying lymphoid organ functioning. Stromal components (epithelial cells or fibroblasts) become connected with macrophages bearing certain antigens. Motile immune cells bearing antigen-specific receptors (T- and B-cells) make contacts to stromal components. Hence, both stromal components and motile cells exert strict immunobiological specificity collectively comprising a distinct level of structural organization. It is worth mentioning that functioning of secretory organs becomes involved in activity of immune system. At some stage, structural components of secretory organs may consist of lymphoid cells and create novel structural assemblies [2, 3]. Breast colustrum and milk contain a great amount of broad spectrum cells. The latter consist of epithelial cells, lymphocytes, lactocytes, myoepithelial cells, macrophages, and neutrophils. Major cellular components in colustrum and subsequent breast milk are presented by macrophages, neutrophils and lymphocytes. Neutrophilic leukocytes function in body defense against fungal and bacterial infections. Amount of

such cells is specifically elevated in colostrum period (up to 50% total composition), whereas in mature breast milk it declines (down to 5% total cell count). Lymphocytes in the breast milk and colostrum being the major cell type of immune system execute cellular and humoral immunity. Mainly, they are presented by T-, B- and NK-cells. Breast milk T-cells produce a whole range of immunoregulatory proteins such as interferons, interleukins, tumor necrosis factor-α. Such cells display immunological memory able to account for maternal immunocompetence [1, 11]. While preparing to lactation, lumen of mammary alveoli becomes filled up with leukocytes, which are removed from the mammary gland with the first portions of secreted colostrum entering neonatal offspring digestive tract. Leukocytes comprise up to one the third of the colostrum volume. Upon entrance, active immune colostrum lymphocytes activate neonate cellular immunity. Upon that, some role may be played by lymphokine trafficking [4]. Being in the digestive tract, maternal cells undergo no degradation, as they might be found in the intestinal mucosal smears by electron microimaging. Microphages, T- and B-cells enter the offspring gut lymphoid tissues via intercellular spaces and hand on antigen-specific immune receptors to neonatal prolymphocytes to arm with potential to recognize foreign non-self substances. Neonatal prolymphocytes after acquiring such biochemical cues and undergoing a multi-step life cycle convert into offspring T- and B-cells. However, some time is required for completing their full maturation and accumulation [9]. Not later than an hour after birth, neonates receiving a first portion of colostrum are featured by elevated blood leukocyte count, marked granulocyte phagocytosis against diverse bacteria evidencing about stimulated hemo- and lymphopoiesis.

Such cells are found in the neonatal circulation. In particular, neonatal male calfs contained maternal sex chromatin-marked leukocytes. Sex chromatin (Barr body) is bound to X-chromosome being normally observed in somatic cells solely in female subjects [8, 10]. It raised a question about presence of durable maternally-transferred cellular immunity in neonates. Hence, it posed a need to study maternal immune cells particularly leukocytes exerting resistance to degradation in the neonate digestive tract and able to penetrate across intestinal walls and enter circulation to provide long-term protection as well as contribute to establishing host own immunity.

Objectives and Tasks

Our study was aimed at revealing immune cell types in breast colostrum and neonatal gut lumen as well as their penetration across intestinal mucosa and detection in peripheral blood and bone marrow after colostrum feeding. The task of the study was to examine cell composition in immune organs of mouse pups after colostrum feeding. For this, there were investigated mouse organs playing a crucial role in whole body immune reactions: red bone marrow and Peyer's patches. In addition, peripheral blood was also collected for analysis.

Materials and methods

The study was conducted with two-day-old inbred mouse male pups after colostrum feeding. Mice were reared at standard conditions in animal facility. All procedures were performed in accordance with requirements for humane use of laboratory animals. Mouse Peyer's patches are found in the ileium as clustered elongated spots of lymphoid tissue. Tissue smears were obtained by attaching dissected Peyer's patches to water-washed and ethanol-ether-defatted glass slide. Red bone marrow was collected as follows: femoral bone was isolated from adjacent muscles and cracked in the middle to collect small portion of bone marrow by tapping onto a glass slide. Peripheral blood was sampled by cutting off tail end. Bone marrow, Peyer's patch and peripheral blood smears were stained according to the Pappenheim method using ready-to-use May-Grunwald solution and liquid Romanowsky–Giemsa dye. Next, tissue smears were air dried and put into sample cell containing May-Grunwald solution for 3 min. After that, smears were washed out with distilled water and put into sample cells containing dissolved Romanowsky–Giemsa dye for 20 min. After staining, smears were washed out with diluted water and air-dried. Sample imaging was performed by using immersion optical microscopy (objective × 100). Mouse pup peripheral blood samples were air dried and stained with Romanowsky–Giemsa dye: work solution was prepared ex tempore based on taking 1 dye drop per 1 ml distilled water. Staining of samples was performed for 20 min which were subsequently washed out with distilled water and air dried. Sample imaging was performed by using immersion optical microscopy (objective × 100).

Sample ultrastructure was analyzed by using fragments of certain regions of the digestive tract straightened on template and fixed with 1.5% glutaraldehyde in 0.1M cacodylate buffer. Transmission electron microscopy was performed after samples were postfixed in 1.5% osmium tetroxide and contrasted by Reynolds method followed by imaging on Hitachi-H-300 microscope. Cells located about at the middle of gastric and intestinal folds were analyzed. However, it is worth noting that no prominent morphofunctional changes were observed while comparing cells at various levels in tissue folds.

Scanning electron microscopy was performed by using samples fixed in glutaraldehyde, dehydrated and underwent CO_2 critical point drying in HCP-2 Hitachi device. Samples underwent gold sputtering followed by imaging in Hitachi-H-300 scanning microscope.

Results and discussion

While conducting transmission electron microscopy of the intestinal epithelial layer, it was found that it contained cellular defense components,

which penetrated from the gut lumen. In particular, lymphoid lineage cells by pushing apart structures in the epithelial layer moved across its apical and basolateral membranes thereby bypassing natural physiological and anatomical barriers while preserving own morphological integrity (Figure 1).

Ultrathin sections of mouse pup intestine revealed structurally diverse immune cells. In particular, it was shown that lymphocytes exerted heterogenous size and ultrastructure. It may be accounted for by varying maturation degree, life span as well as diverse functions and antigen-specificity. Most of circulating lymphocytes are featured with a large nucleus, high nucleoplasmic ratio, and underrepresented cytosolic organelles. Cells structurally resembling small lymphocytes were most common in the blood stream.

While examining small mouse pup intestinal microsections by using electron microscopy it was found that they contained cells corresponding to small dark lymphocytes. Such cells detected by us had round, sometimes notched, nucleus with heterochromatin presented as large clumps. Sometimes, heterochromatin was distributed homogenously across entire nucleic region. The cell nucleus is located at the center being surrounded by a thin rim of fine-grained light cytosol as well as a nucleolus or its fragments (Figure 2). Nuclear membrane is clearly observed, with a dense chromatin located nearby. The cells contain small amount of cytosol and organelles, with few large spherical mitochondria and pinocytotic vesicled were found. Endoplasmic network is observed as small isolated sheets. In addition, cytosol also contained large amount of ribosomes.

In addition, intestinal ultrathin sections were shown to contain cells ultrastructurally resembling plasma cells, which are clearly differed from other immune cells. Mature plasma cells possess eccentric round nucleus, with small ring-shaped nucleolus. Chromatin is located peripherally close to the nuclear membrane. Ribosomes are assembled into polysomes found as clustered rosettes. Mitochondria were of spheric shape. Golgi apparatus was large that surrounded centrosome and formed extended perinuclear structure. Extensively developed granular endoplasmic network was prominently observed inside cells (Figure 3).

In addition, we also found macrophages in the intestinal mucosa and submucosa. Macrophage beanlike or irregular nuclei were of average size and contained chromatin clumps, whereas cytosol contained large amount of lysosomes, phagosomes and pinocytotic vesicles. In contrast, amount of mitochondria, endoplasmic network and Golgi apparatus was decreased. While analyzing intestinal smears from newborn mouse pups by light microscopy, we found on the surface of intestinal mucosa and intestinal sections a great number of leukocyte lineage cells (lymphocytes and neutrophils) with preserved functional morphological structure.

Further investigation of intestinal microsections with electron microscopy allowed us also to visualize maternal immune cells, which attach as if pushing apart epithelial layer and penetrate into mouse pup intestinal wall while preserving own morphological integrity (Figure 4).

Ultrathin intestinal sections contain cells ultrastructurally resembling plasma cells bearing small eccentric nucleus and large Golgi apparatus. Clustered ribosomal rosettes are found in the cytosol almost entirely covered with well developed enlarged granular endoplasmic network. While examining mouse pup blood samples, it was found that leukocytes contained Barr bodies. Intensity of X-chromatin-specific chromatin staining was higher compared to

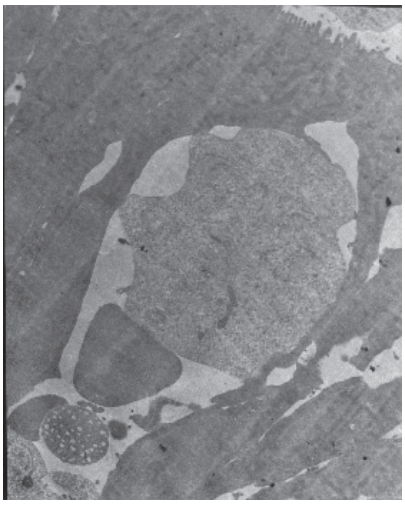

Figure 1. Transmission electron microscopy of the intestinal epithelial layer in newborn mouse pup after colostrum feeding. A maternal leukocyte penetrating across intestinal epithelial layer is visualized

Note. Magnification × 3,000.

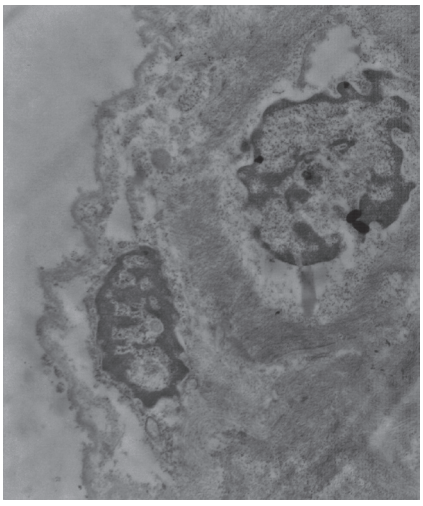

Figure 2. Transmission electron microscopy of the intestinal epithelial layer in newborn mouse pup. Maternal leukocytes penetrating at the site of intestinal serous membrane are depicted

Note. Magnification × 3,000.

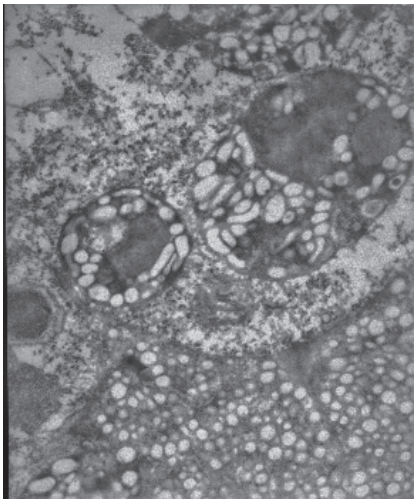

Figure 3. Transmission electron microscopy of plasma cells inside intestinal epithelial layer in newborn mouse pup
Note. Magnification × 3,000.

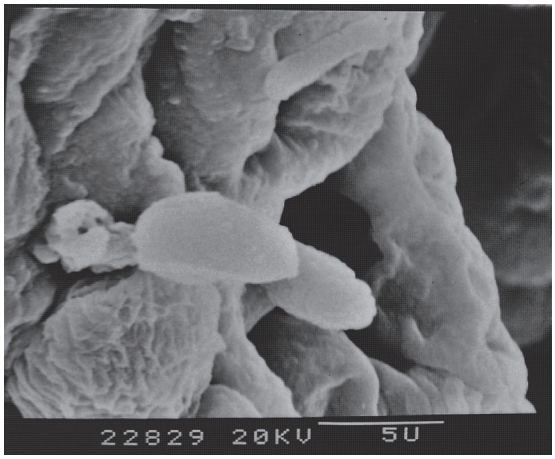

Figure 4. Scanning electron microscopy of intestinal surface. Maternal leukocytes penetrating into the intestinal epithelium are depicted
Note. Magnification × 3,000.Scale – 5 μm.

other chromatin areas, whereas any form of clear-cut sex chromatin-containing bodies was identified.

While conducting morphology analysis of bone marrow leukocyte lineage cells and gut lymphoid assemblies, we found sex X-chromatin-bearing immune cells (Figure 5).

Thus, our study allowed to unravel that maternal leukocytes with colostrum intake entered newborn mouse pup intestine and were able not only to cross the gut epithelium and enter the circulation, but also traffick towards the central and peripheral lymphoid tissues and subsequently take part in formation of antigen-specific T and B-cells, interact with antigen-presenting cells by contributing to establishment of neonatal immune system in mouse pups and durable long-lasting immunity.

A potential for colostrum immune cells to enter newborn subject circulation is verified by detecting natural maternally-derived specific marker called sex chromatin. In particular, this technique is based on examining structural inclusion located inside cell nuclei – sex X-chromatin, which is lacked in male-derived cells. Hence, such approach was applied to identify sex chromatin-tagged leukocytes in male newborn subjects. Detecting maternal colostrum-derived cells in neonatal intestinal wall and circulation revealed that they comprised around 25, 1 and 70% total cell composition in the peripheral blood, lymph and gut, respectively. Undoubtedly, colostrum-derived leukocytes play a pivotal role in establishing local and systemic immunity in newborn animals. Previous evidence demonstrated that after colostrum feeding blood leukocyte count in animal offsprings becomes elevated primarily due to thymus-derived lymphocytes. Newborn lymphatic system undergoes certain reorganization to gain immune-related education. At least, it may be illustrated by a long established fact when granular leukocytes

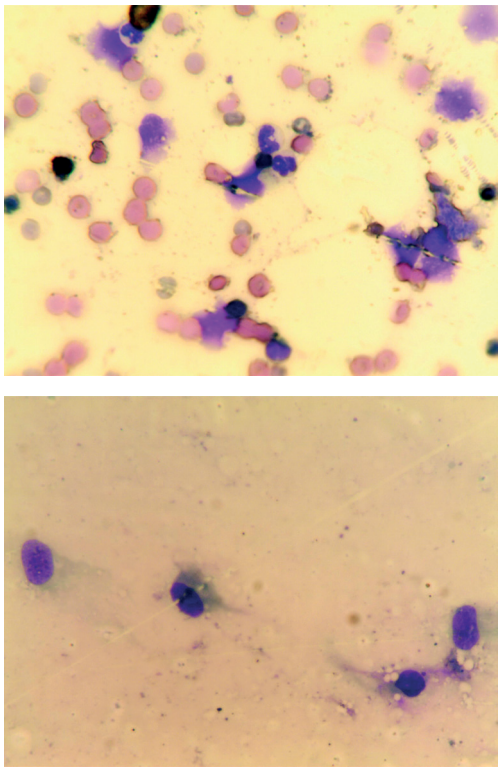

Figure 5. Barr bodies in the cells of red bone marrow smears from newborn pups

such as eosinophils and neutrophils emerged in digestive tract mucosa in late-gestation fetuses. Such cell types appeared earlier and became enriched in high numbers in the small intestine, especially in the ileum. Moreover, this anatomical region in late-gestation sheep and swine fetuses was found to contain unique clumpy leukocytes. Their generation begins with migration of leukocytes from mucosal connective tissue into the space between epithelial

cells at the villus base and crypts. Such lymphocytes lose basophilic properties in the protoplasm and start producing specific eosinophilic granules, which is paralleled with increased cell size. Moreover, it was shown that such granules lie free between epithelial cells. In addition, it also resulted in describing lymphocyte efflux from inter-epithelial space into mucosal connective tissue. However, plasma cells emerge in sheep and swine intestinal mucosa before birth, whereas in cattle they were detected postnatally, perhaps due to colostrum intake containing a marked immunoglobulin amount [12].

During lactation, maternal immune system is extremely oriented at defending and maintaining neonatal immune status within the first days of life. It is accomplished via immediate control over neuroendocrine regulation. All nutrients and immunoglobulins necessary for body growth and development are provided to neonates via colostrum and breast milk. However, it should be taken into consideration that immunoglobulins as defense molecules enter colostrum only at the onset of lactation period. Later, their level and potential to cross gut epithelial barrier profoundly decline. However, efflux of maternal cells into colostrum and mature breast milk is sustained over entire lactation period, which we showed to be stimulated via oxytocin. Hence, the data of our studies allow to assume that maternal cellular defense components serve as constituents both in colostrum and breast milk immunity. As a result, a newborn subject becomes armed with durable and long-lasting immunity upon natural breastfeeding [5, 6, 7].

References

1. Dementyeva Yu.N. Immunological aspects of breastfeeding. *Russian Bulletin of Perinatology and Pediatrics, 2015. Vol. 60, no. 4, pp. 19-24.* (In Russ.)
2. Hurley W.L. Mammary gland growth in the lactating sow. *Livestock Product. Sc., 2001, Vol. 70, no. 1/2, pp. 149-157.*
3. Hurley W.L., Kim S.W. Characterization of mammary gland development in pregnant gilts. *J. Anim. Sci., 2006, Vol. 84, no. 3, pp. 579-587.*
4. Kolyakov Ya.E. Veterinary immunology. Moscow: Agropromizdat, 1986. 227 p. (In Russ.)
5. Markowska-Daniel I., Pomorska-Mól M. Shifts in immunoglobulins levels in the porcine mammary secretions during whole lactation. *Bull. Vet. Inst. in Pulawy, 2010, Vol. 54, no. 3, pp. 345-349.*
6. Markowska-Daniel I., Pomorska-Mól M., Pejsak Z. Dynamic changes of immunoglobulin concentrations in pig colostrum and serum around parturition. *Pol. J. Vet. Sci., 2010, Vol. 13, no. 1, pp. 21-27.*
7. Mielke H. Geschichtliches und Grundlagen der immunobiologisehen Bezichungen zwischen Muttertier und Frucht beim Rind. *Mh. Vet. Med., 1979, Bd. 34, no. 6, pp. 217-223.*
8. Panova N.A., Skopichev V.G., Polistovskaya P.A. Immune cell composition and cell-related structure in mouse mammary gland during lactation and physiological quiescence. *Regulatory Issues in Veterinary Medicine, 2017, no. 3, pp. 193-196.* (In Russ.)
9. Plyashchenko S.I., Sidorov V.T., Trofimov A.F. Breeding and farming healthy calves. Minsk: Uradzhay, 1990. 250 p. (In Russ.)
10. Pogodaeva P., Skopichev V., Alistratov F., Dushenina O., Vasilyeva S., Vasilyev R., Pilayeva N. Cells of immune memory in mice in the colostrums. *Journal of the Reproduction in Domestic Animals, 2019, Vol. 54, no. 3, p. 103.*
11. Skopichev V.G., Maksimyuk N.N. A milk: a study guide. St. Peterburg: Prospekt Nauki, 2011 368 p. (In Russ.)
12. Skopichev V.G., Prozorovskiy V.B. Mammary gland immunobiology and lactation performance. Beau Bassin: Lap LAMBERT Academic Publishing, 2018. 328 p. (In Russ.)

Авторы:

Панова Н.А. *– к.б.н., доцент кафедры биохимии и физиологии ФГБОУ ВО «Санкт-Петербургский государственный университет ветеринарной медицины», Санкт-Петербург, Россия*

Скопичев В.Г. *– д.б.н., профессор кафедры физиологии лечебного факультета, Институт медицинского образования ФГБУ «Национальный медицинский исследовательский центр имени В.А. Алмазова» Министерства здравоохранения РФ, Санкт-Петербург, Россия*

Authors:

Panova N.A., *PhD (Biology), Associate Professor, Department of Biochemistry and Physiology, St. Petersburg State University of Veterinary Medicine, St. Petersburg, Russian Federation*

Skopichev V.G., *PhD, MD (Biology), Professor, Department of Physiology, Faculty of Medicine, V. Almazov National Medical Research Centre, St. Petersburg, Russian Federation*

Поступила 15.03.2021
Отправлена на доработку 03.06.2021
Принята к печати 09.06.2021

Received 15.03.2021
Revision received 03.06.2021
Accepted 09.06.2021