



Research article

Calf demand provision by mammary gland secretion during the first decade of post-natal development



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ABSTRACT

The article highlights the experimental results on calf demand provision by mammary gland secretion during the first days of post-natal development. Changes in mammary gland secretion content during the transition process from colostrum to natural milk were showed. Lactoferrin level changes were demonstrated. Population-based composition of colostrum somatic cells was determined. A hypothesis concerning apoptosis role of the somatic cells and neutrophils' burst was presented. The present study highlights calf provision with mammary gland secretion during the first 10 days of the postnatal development.

1. Introduction

Increasing the vitality of the organism in early postnatal ontogenesis remains one of the crucial problems in modern biology. Special attention is paid to metabolic processes of the animal organism in different periods of a productive cycle, i.e. the mother's "body – the fetus – the newborn animal." Early postnatal mammalian ontogenesis is characterized by the development of oxidative stress and adaptive responses to new conditions of existence [1]. Satisfaction of the physiological needs of the newborn occurs owing to the nutrients received from the mother, with colostrum and milk.

Colostrum is a multicomponent and multifunctional substance containing essential nutrients (lipids, proteins, lactose, free amino acids) and biologically active substances: immunoglobulins, antibodies, proline-rich polypeptides (PRP), lactoferrin (LF), glycoproteins (proteoglycans, trypsin inhibitors), lactobumines, cytokines: interleukin 1 and 6, interferon and lymphokines; growth factors: epithelial (EGF), insulin-like 1 and 2, transformed A and B, platelet-derived growth factor (PDGF); vitamins and minerals (macro and microelements) [2, 3, 4, 5, 6].

Colostrum provides two very important functions, namely nutritious and protective. The first is provided with a high content of nutrients and biologically active substances [7]. Colostrum is responsible for growth factors of the intestinal epithelium, inhibition of lymphocyte activity, stimulation and suppression the growth of microorganisms' colonies [8].

Historically, the attention of the researchers was directed to colostrum-induced passive immunity of calves in their first days of life, since the structure of the ruminant's placenta (the syndesmochorial type) does not pass protective antibodies circulating in the vascular system of the mother into the bloodstream of the fetus, thus depriving newborn calf of specific antibodies (immunoglobulins). The first study describing the introduction of immunoglobulins into the body of calves with colostrum was dated to 1892 by P. Ehrlich [9] and was experimentally proved by P.E. Howe in early 1920s.

However, the effect of colostrum is not limited to providing nutrients and development of immune status, but more multifaceted. In this regard, the assessment of regularities and formation of the theory of newborn organism' adaptation to postnatal conditions fades into insignificance to the necessity to determine the most general principles of

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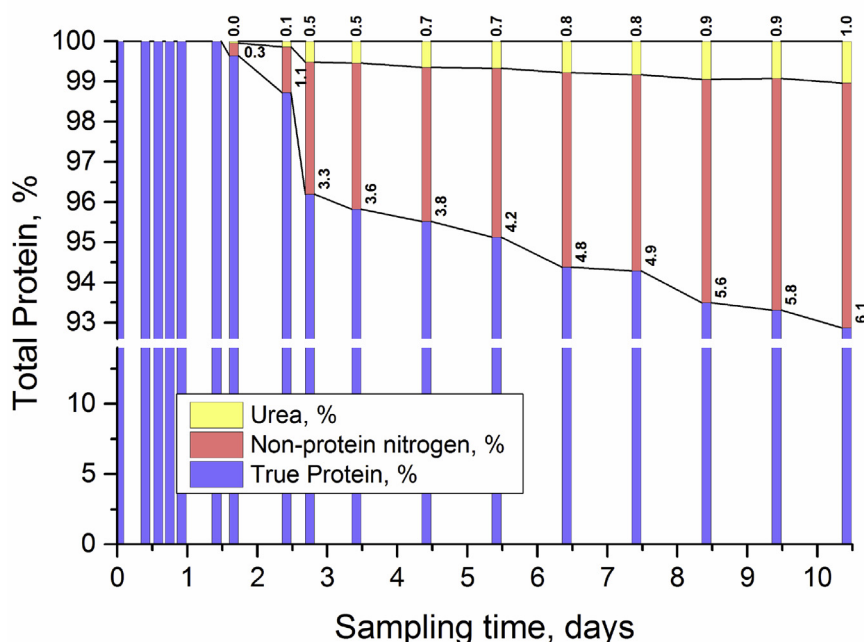


Fig. 1. Dynamics of changes in total protein composition in the first 10 days after calving.

biological systems' functioning to form the basis for the development of theoretical knowledge.

Proceeding from the above and the complexity of alteration of regulating mechanisms during the transition from embryonic to postnatal development, we aimed to study the dynamics of changes in chemical composition of colostrum, its biologically active components, the totality and functional significance of isolated cytotubules, the prooxidant and antioxidant status of the secretion of a mammary gland in the initial stage of lactation.

2. Materials and methods

The plan of research was reviewed and approved by Independent

Ethics Committee of the Federal State Budgetary Institution of Science Center for Theoretical Problems of Physico-Chemical Pharmacology of the Russian Academy of Sciences. All the procedures connected with the collection of biological materials were conducted in accordance with recognized ethical standards, norms and regulations of Russian Federation and international laws.

The main parameters of selected samples (dry matter, fat, protein, dry skim milk residue, lactose) were examined using Bentley-150 milk analyzer. For this, the samples were placed in 50 ml Falcon-type polypropylene tubes, from which a sample was taken using an autosampler. The analysis was carried out using the built-in calibration equations. Crude ash content was determined by dry ashing by combustion at 525 °C for 6 h. The milk freezing point was determined conductometrically.

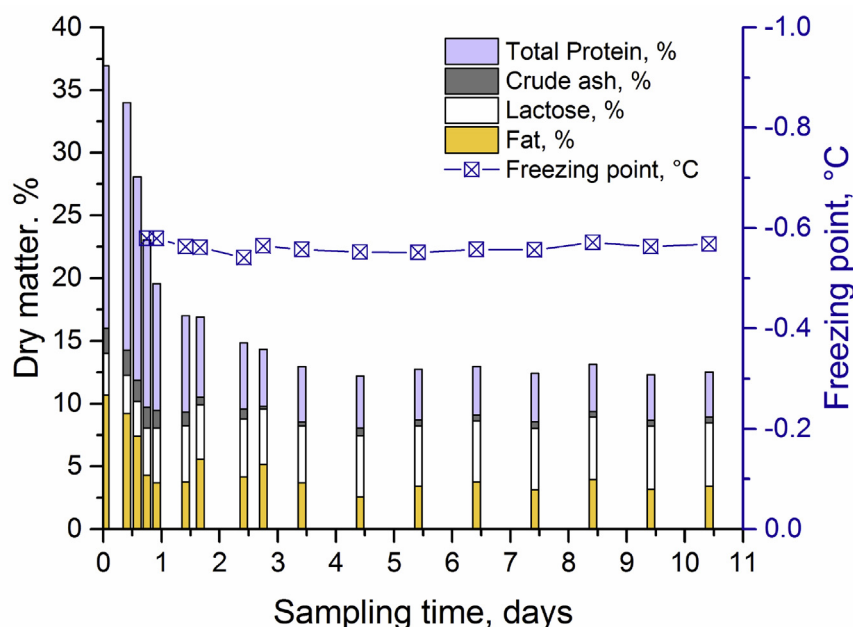


Fig. 2. Evolution of the chemical composition of the mammary gland secretion during the first decade after calving.

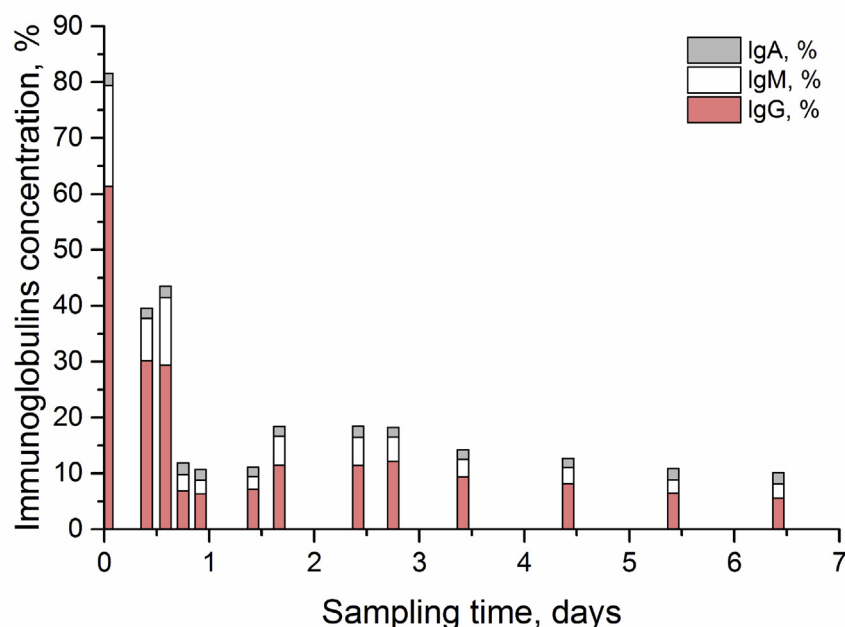


Fig. 3. Dynamics of changes of immunoglobulins content in the mammary gland secretion of cows.

Contamination of the mammary gland secretion by somatic cells at the initial stage of lactogenesis was estimated using Somacount 150 analyzer by the method of flow-laser cytometry. For this, the samples were placed in 50 ml Falcon-type polypropylene tubes, from which they were taken automatically and mixed with a fluorescent dye solution. This dye disperses the globules and stains the DNA in the somatic cells. Then aliquot part of the stained suspension was injected into a laminar stream of carrier fluid for further analysis and detection.

The micronutrients of the cellular secretion were painted (Unna-Pappenheim method) in order to observe changes in the nucleus (picnose, fragmentation, vacuolization), in the cytoplasm (granularity and vacuolisation), thiozsis, and cell size dynamics. The total number of immunoglobulins in the secretion of the mammary gland was determined by colostomyram, separate classes of immunoglobulins and lactoferrin were evaluated by the method of immunoassay analysis according to the generally accepted method [10]. The content of trace elements was measured using atomic absorption spectrometer AAS-30. The samples were first digested. An aliquot of samples, obtained after digestion, was injected into the atomic absorption spectrometer with the help of an auto-sampler, and the elemental concentrations were obtained using an external calibration curves.

3. Results and discussion

Figs. 1 and 2 show the dynamics of changes in secretion of mammary gland main components, namely colostrum, transitional colostrum-normal milk secretion and whole milk (10th day of lactation).

It was found that during 10 days of lactation, the level of true protein gradually decreases almost 7 times, while the level of non-protein

compounds and urea increases 10 and 13 times respectively (Fig. 1). The content of lactose in milk also increases more than 1.5 times compared to initial values. At the same time a noticeable reduction of dry matter in the secretion of the mammary gland can be observed (Fig. 2). In other words, the composition of main components providing both energy and biological value of colostrum and milk constantly changes during the initial 10-days (lactation) period.

The data on freezing point of colostrum (Fig. 2) indirectly characterize its naturalness and allows estimating the quantity of monovalent charged ions, determining the electrical conductivity of gland secretion [11]. Using this parameter, it is easy to determine the moment when mammary gland reaches constancy in functioning. This point is characterized by decrease of both sodium and chlorine ions level.

The maximum content of immunoglobulins in milk is observed on the first day of lactation, when they can penetrate the epithelium of the intestinal tract without any interference (Fig. 3). Later, the immunoglobulins content rapidly decreased during the transition from the actual colostrum fraction to the secretion of natural milk. The reasons for this process, its significance and the relationship with plasma cells will be discussed below. The results of the study on trace elements' content in colostrum are presented in Table 1.

Before discussing the obtained results, it should be noted that with transition to the pulmonary type of respiration, in addition to oxygen in its different forms, the mammalian organism receives with the first portions of colostrum a sufficiently large amount of free radicals of peroxides and hydroperoxides. In the early postnatal period, the activation of oxidative processes in cells of the hemopoiesis system is accompanied by the intensification of lipid peroxidation (LP) processes with the accumulation of LP products in bone marrow, blood and lymphoid cells

Table 1
Changes in trace elements' content in colostrum with time, mg·kg⁻¹ of natural sample.

Sampling time, hours	Cu	Mn	Zn	Fe	Cd	Total	% of Crude Ash
0.67	0.020	0.084	6.90	2.00	0.040	9.04	0.045
22	0.024	0.081	6.64	1.97	0.039	8.75	0.062
34	0.029	0.078	6.00	1.80	0.031	7.94	0.074
82	0.038	0.068	5.43	4.19	0.021	7.05	0.041
Difference between samples taken at 0.67 and 82 h	0.018*	0.016*	1.47*	2.19*	0.019*	–	–

* Absolute difference between the first (0.67 h) and the last (82 h) samples when $p \leq 0.05$.

during the first days after birth. In the same period an increase in the activity of enzymes of the antioxidant system (SOD, glutathione peroxidase) can be observed in the cells of bone marrow, blood and lymphoid organs [12, 13]. In a whole it leads to an increase in the normalization of the content of LP products.

Breastfeeding during the first 2 h after calving is characterized by a high content of immunoglobulins and somatic cells, which have a high level of LP products, as the concentration of peroxides increases under the conditions of a "neutrophil burst". It is believed [14, 15, 16] that both "burst" and apoptosis are regulated and activated by proteins of acute phase and activation of the cascade of apoptotic proteases (caspases). In addition to bacteriostatic factors such as lactoferrin (LF), mumidase, lactoperoxidase, lysozyme, somatic cells under lysis conditions can also be considered as an antimicrobial factor. Perhaps there are several levels of protection, both colostrum and gastrointestinal tract of infant, from pathogenic microflora and its toxic metabolites (endotoxins) e.g. tayhoic acid, peptidoglycans, polysaccharides.

It is known [5, 6, 17] that colostrum has a high level of lactoferrin, whose main function is to maximally bind Fe ions, being the integral part of reproduction of microorganisms. However, a low level of Fe in colostrum and milk cannot meet the needs of a newborn organism. Fe reserves in the body at birth along with the amount found in colostrum and mother's milk do not cover needs in it. As a result, the body of the young faces its deficiency, which leads to anemia of various geneses, including iron deficiency. It is possible that Fe received by the newborn organism from colostrum can be associated with lactoferrin and thus not absorbed by the body [13]. Losses of young animals for this reason may be greater than infectious diseases [18]. Therefore, the technology of growing young animals is not possible without the use of Fe-containing additions. It should be noted that according to the hypothesis [19], free radicals can activate the center of satiety and, thus, cause a loss of appetite and feed depression. In this regard, forced (through the probe) introduction of colostrum to many mammals in the first days after birth can be necessary.

Normally, milk contains 2–4 mg kg⁻¹ of iron. From this amount, 10–30% is associated with fat fractions, mainly with an outer membrane around the fat globule. A small fraction is bound to casein, the other is contained in serum and is mainly associated with LF [20, 21], as well as with low molecular weight ligands [22]. Thus, about 30% of iron contained in milk is associated with LF, but only 6–8% LF is saturated with iron, so the majority of LF is in apo form [23].

The acute problem nowadays is a development of enzyme-imitating substances that can increase the antioxidant and immune status, normalize metabolic processes in tissues, and restore the structure and

function of organs and systems. It is known that the absorption of IgG from colostrum is mediated by the activity of intestinal penicillois, which is the most intense in the first 30 min, and continues for up to 24 h. Recent studies [24] show that the introduction of additional essential micronutrients, namely selenium in colostrum, directly activates the physiological function of pinocytosis in the intestinal epithelial cells and accelerates the rate of immunoglobulins' accumulation of in the blood by 20% more. It was also shown that introducing additional essential micronutrients provides their required amount, contributes to the development of the immune system and increases the level of IgG in blood plasma of newborn calves.

While a considerable amount of research is devoted to studying the structural components of colostrum and their functions, the interest in clarifying the role of this substance does not diminish even now, reflecting the importance of colostrum in the life of a young organism. However, with the accumulation of experimental data and research results, an imbalance in favor of works devoted to humoral factors against its cellular components contained in colostrum can be noticed. Therefore, one may suggest that the complex organization of colostrum mechanisms is still underdeveloped. This is especially evident in the study of physiological mechanisms of immunoactive components transfer from mother to a child. And while the interest in immunoglobulins and the factors of non-specific protection of colostrum is relatively constant, studies of colostrum cells appear much rare. Numerous studies conducted with different animals on the whole organism, isolated organs and their fragments, as well as individual cell studies allowed to identify the levels at which cell interactions may occur both in the mammary gland and in the mucosal-related lymphoid tissue. Fig. 4 shows the dynamics of changes in content of somatic cells and lactoferrin in the secretion of the mammary gland of cows. Only animals with no evidence of a clinical or subclinical mastitis were included in the experiment.

The level of somatic cells decreased almost 12 times in the first 250 h of lactation. The concentration of lactoferrin (the dominant iron binding protein) remains on practically the same level. This finding contradicts the previously reported data that LF concentration in "true" raw milk is 2–9 times less compared to colostrum. In our study, the constancy of LF concentration can be explained by the fact that the detected level of IgA (Fig. 2) was relatively low compared to other fractions. The bactericidal function of IgA is realized only in the presence of lactoferrin, which has a bacteriostatic effect on pathogenic and opportunistic bacteria. The mucous membrane of the newborn calf is able to produce lactoferrin only at very low concentrations.

Thus, colostrum is a unique, very complex product that is synthesized in the mammary gland during the first 5–7 days of lactation, although a

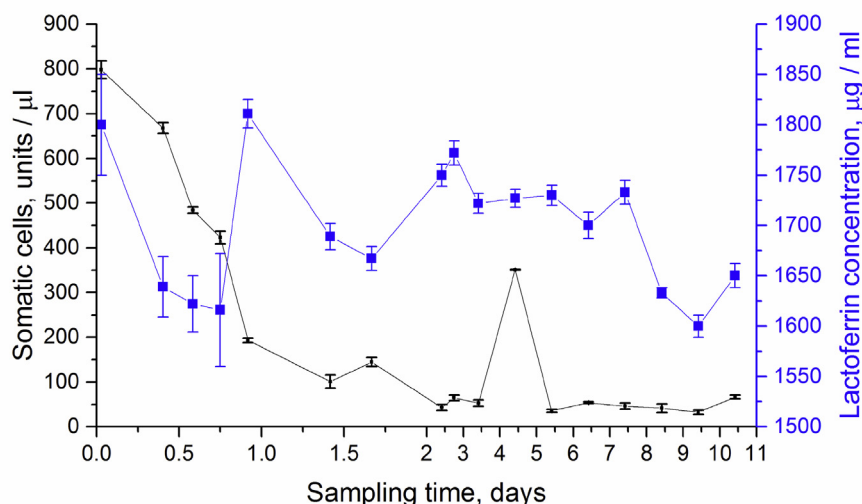


Fig. 4. Dynamics of changes in content of somatic cells and lactoferrin in the mammary gland secretion of cows.

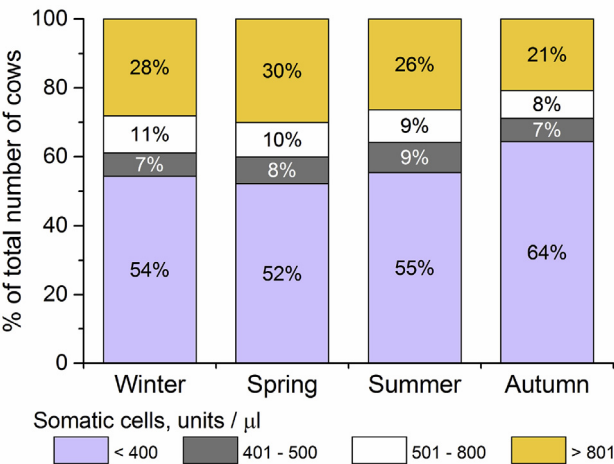


Fig. 5. The distribution of milk samples by the content of somatic cells.

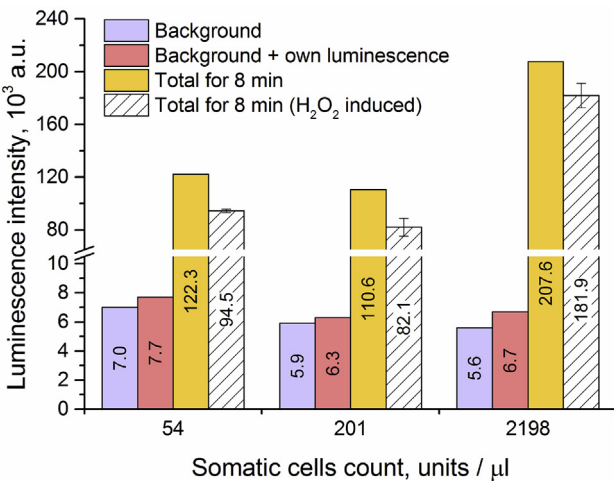


Fig. 6. The influence of H₂O₂ on chemoluminescence intensity of milk containing different number of somatic cells.

liquid similar in composition begins to exude 2–3 days before calving. It is known [25] that the main part of colostrum proteins consists from immunoglobulins. High levels of leukocytes and immunoglobulins

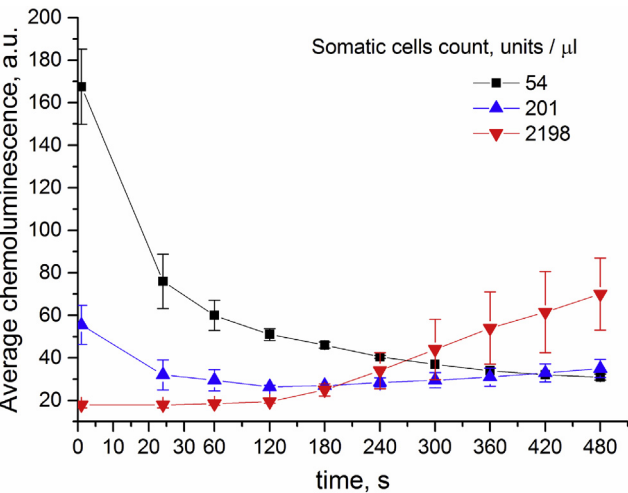


Fig. 7. Dynamics of H₂O₂ induced biochemiluminescence of milk depending on somatic cells count.

Table 2

Structure of somatic cells' subpopulations depending on their concentration in milk.

Somatic cells, units/μl	98–100	180–200	380–420	580–620	780–820
Segmental neutrophils	36	21	56	60	72
Total	39	25	58	60	72
% of total	92.3%	84.0%	96.6%	100%	100%

provide its protective function. Immune globulins accumulate in the mammary gland in two ways. The first way is when they are brought into the mammary gland with blood. In this case, the titre of antibodies in colostrum depends on their concentration in the blood of the mother. The second way is when the antibodies are produced in the mammary gland in response to the penetration of the antigen (dialectical immunity).

It was found that the mammary gland contains a large number of plasma cells that produce immune globulins. Many researchers [26] proved that introduction of antigens directly into the mammary gland allows obtaining high titers of antibodies both in colostrum and milk. Accumulation of antibodies in the mammary gland and their appearance in the colostrum is observed immediately before delivery.

Leukocytes appear in the mammary gland long before lactation. They are involved in clearing the lumens of the alveoli with proteolytic enzymes, oxidase, or AFC products [27]. Moreover, the inhibition of the regional lymph node activity significantly affects morphological processes and prepares development of the alveoli structure. There is a significant increase in the clone of T-lymphocytes and plasma cells in the mammary gland during the colostrum period. In addition, there is an

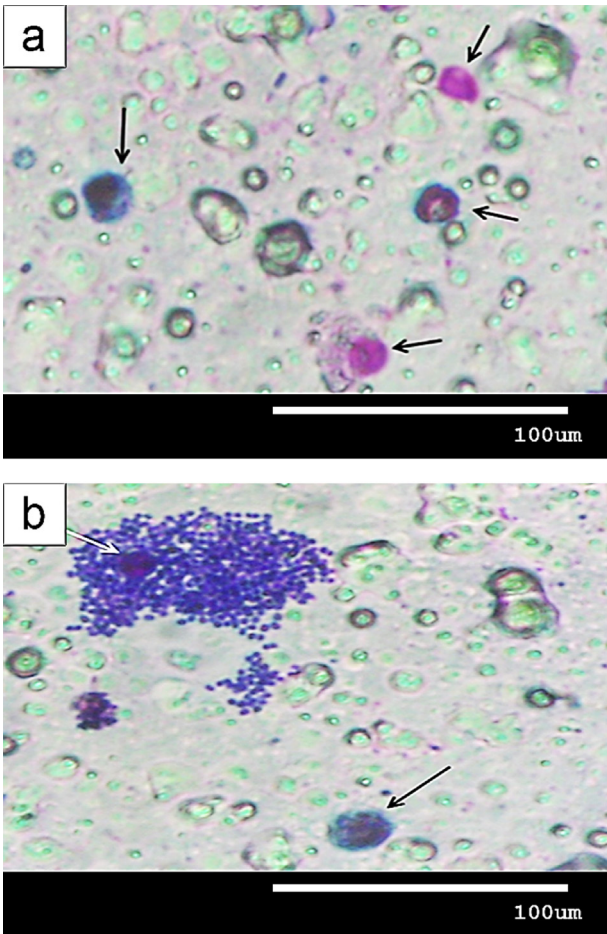


Fig. 8. Cytological study of somatic cells population in a milk sample (SC = 180–200 units/μl): a) Neutrophils; b) Neutrophils (white arrow) and lymphocytes (black arrow).

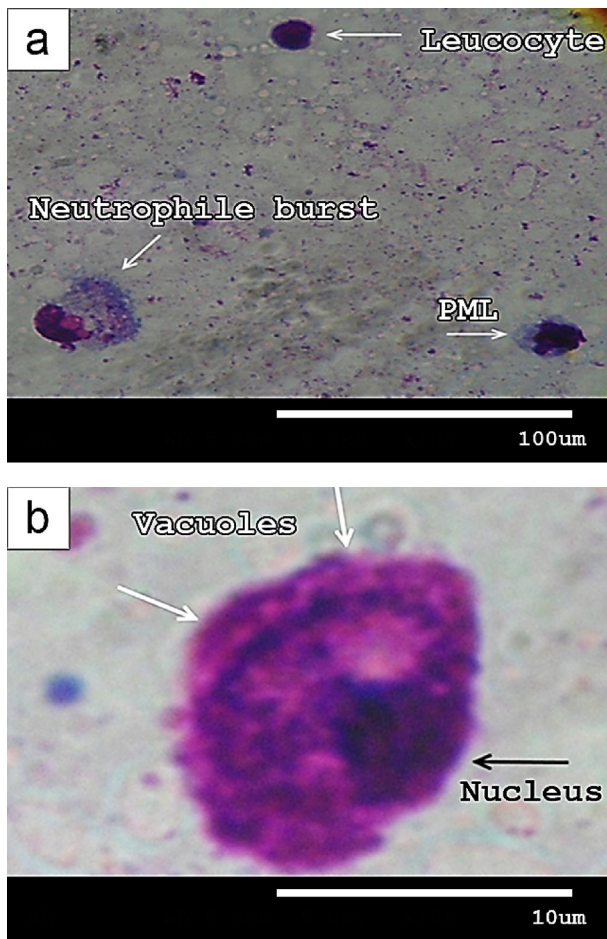


Fig. 9. Cytological study of the somatic cells population in a milk sample (SC = 580–620 units/ μ l): a) Somatic cells; b) Neutrophil vacuolation.

increase in neutrophils and monocytes number.

Cellular elements and immunoglobulins of colostrum enter the gastrointestinal tract of the newborn and participate to the formation of immune defense. The time range for transmitting maternal antibodies to newborn is different. In general, the process of migration through the intestinal wall and appearance in blood takes about 3–6 h [28]. Authors [29] reported that lymphocytes are able to influence the intensity of migration and proliferation of hematopoietic cells, as well as the direction of their differentiation. In addition, there are indications of the influence of T - lymphocytes on the migration of stem cells from the bone marrow and intensifying the granuloid type of their differentiation. The granuloid differentiation of stem cells is necessary for the formation of phagocytic blood cells and tissue macrophages.

It is known [10] that colostrum intake greatly increases the amount of leukocytes in the blood due to obtaining lymphocytes, total protein and immunoglobulins. The study of authors [30] showed the possibility of immunoglobulins (IgA) synthesis by leukocytes of colostrum. The observed even *in vitro* synthesis of immunoglobulins indicates the active participation of cellular elements in the formation of humoral immunity of the newborn. All these data allow shedding more light on the entire complex of interactions occurring between immune system of mother and the newborn.

One more important aspect receiving much attention is milk contamination by somatic cells during lactation and its stages. This factor is considered as a health indicator of udder and indirect index of economic performance, because high concentrations of somatic cells in dairy raw materials decrease the quality of milk and dairy products [31, 32, 33]. To evaluate the annual variation of somatic cells in the milk of

pedigree cows depending on the season, totally about 35 000 samples of milk were tested during a 5-year's period. The influence of paratypic and genetic factors, technologies and conditions for keeping animals were taken into account. The total number of cows in the experiment varied, namely 7575 in winter, 8919 in spring, 11337 in summer and 7515 in autumn. The distribution of milk samples by the content of somatic cells is presented on Fig. 5.

To the date, there are a sufficient number of contradictions concerning the interpretation of the functional value of somatic cells, the role of their apoptosis and signaling mechanisms [34, 35].

For further investigation of this phenomenon, we performed a biochemiluminescence study of these cells. In particular, the interconnection between an intrinsic and H_2O_2 induced ultra-weak glow of milk with different concentrations of somatic cells was investigated. Tested samples contained 54, 201 and 2198 thousands units per milliliter. The results are presented on Figs. 6 and 7.

The dependence between the somatic cells concentration and milk own ultra-weak luminescence, the time of induction of flashes and their amplitudes, as well as the total light sum of the reaction were established. The analysis of biochemiluminescence curves revealed that the process induced by H_2O_2 in milk with high content of somatic cells significantly differs by direction, deviation and inclination from specimens with the physiologically specified level of somatic cells in milk. However, a further study on influence of free radical and antioxidant reactions in milk contaminated with somatic cells with an increased number of specimens is needed. In this regard, we investigated changes in the structure of subpopulations of somatic cells caused by changes in their

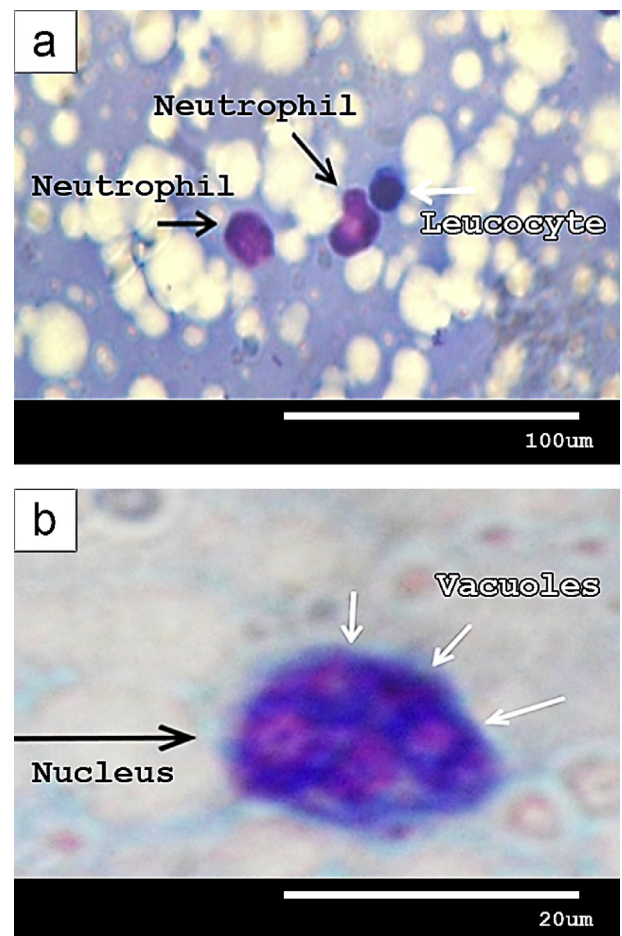


Fig. 10. Cytological study of the somatic cells population in a milk sample (SC = 900–1200 units/ μ l): a) Neutrophils and lymphocytes; b) Vacuolation in neutrophils.

concentration in milk (Table 2).

From Table 2, the increase of somatic cells' content in milk increases the level of polymorphonuclear neutrophils, namely the population of segmental neutrophils. This finding is in good agreement with earlier data [35], the authors showed that polymorphonuclear neutrophils (PMNs) became predominant species with an increase of somatic cells content.

Figs. 8, 9, and 10 demonstrate the interrelation between neutrophil apoptosis and their population in the milk of cows. Neutrophils are capable of apoptosis not only in the gland, but also directly in a secretion, i.e. in the milk or colostrum. This fact was confirmed by cytological studies while investigating certain morphological structures, namely changes in the nucleus (picnose, fragmentation and especially vacuolization), cytoplasm, in the form and size of the cells.

Obtained data indicates that the cell population in colostrum and milk affects the bacteriostatism of the mammary secretion and functioning of the antioxidant and prooxidant balance of the body of calves.

4. Conclusions

A fairly fast variability of the chemical composition and biologically active components of the secretion of the mammary gland during the transition from colostrum to natural milk was shown. In the first 10 days of lactation, the secret of the mammary gland covers the needs of newborn calves with energy and nutrients. Moreover, it plays an important role in the established of immune status and in adaptation responses to new living and environmental conditions.

In the process of adaptation to new living conditions, the crucial role is played by the intake of essential trace elements, which are the basis for the synthesis of metal enzymes. In case the level of nutrient trace mineralization incoming with colostrum is insufficient, iron deficiency anemia of newborn calves can appear.

The existence of multilevel regulatory systems in colostrum can be assumed. In particular, the low content of Fe prevents free radical pathologies caused by free ions. The high concentration of lactoferrin in colostrum ensures binding of ferrous ions and plays a bacteriostatic role by suppressing the growth of Fe-consuming microflora, including pathogenic ones. The presence of somatic cells, specifically polymorphonuclear neutrophils, provides the bactericidal phase of colostrum and milk. The possible simplified scheme is as follows: signal – apoptosis – burst – release of free radicals in the environment – destruction of microorganisms' membranes. Moreover, free radicals in colostrum can activate the development and formation of protective antioxidant mechanisms in the body of newborn calves during the initial period of postnatal ontogenesis.

The results on colostrum's main components dynamics can be used for applied purposes, namely to increase the vitality of newborn calves by feeding with colostrum of a various quality independently or mixed colostrum (convalescent). From the theoretical point of view this study allows assessing bioavailability of the first secretion of a mammary gland.

Declarations

Author contribution statement

Sergei Mikhaylov: Conceived and designed the experiments; Wrote the paper.

Sergii Shapovalov: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Skryl Andrey: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Yelisaveta Cheresheva: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Dibahan Tsomartova: Performed the experiments; Wrote the paper.

Marina Ivanova, Mariia Pavlova: Analyzed and interpreted the data.

Elina Tsomartova, Diana Shapovalova: Performed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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