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THE MORPHOLOGICAL CHARACTERIZATION AND THE ACTIVITY STATES OF THE EPITHELIAL CELLS PRESENT IN THE JENNET COLOSTRUM

Laurenţiu OGNEAN[1]*, Cristina ŞTEFĂNUŢ[1], Alina NĂSĂLEAN[1], Emöke PALL[1], Andreea BUTA[1], Attila HARI[1], Octavia NEGREA[1]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Romania

*Corresponding author e-mail: lognean@yahoo.com

Abstract

The colostrum secretion of different mammalian species contains heterogeneous cellular populations which come from lactiferous structures and from the general circulation, including various types of epithelial cells, respectively leukocytes. During the synthetis and milk-ejection process, in lactation are involved different types of epithelial cells, with a wide morphological and functional diversity. The aim of this research is represented by the cytomorphological investigation of the first jennet colostrum, which was drawn from healthy mammary gland. The colostrum samples were processed in stained slides, using Dia Quick Panoptic method and they were examined at the microscope and they were evaluated by using the milk cytogram technique (Ognean et al., 2011). The conducted studies have highlighted morphological elements and activity states necessary for the characterization of the cellular populations, regarding the morpho-functional description of the epithelial cells. The results revealed a significant percentage of the epithelial cells in the jennet colostrum. In the configuration of this cellular population, we have identified alveolar, squamous and columnar epithelial cells. The alveolar cells have prevailed among the epithelial cells and they have reflected the secretory activity of the alveolar epithelium. The squamous epithelial cells were distinguished due to their predominant polygonal cytoplasm and the punctiform nucleous, while the columnar epithelial cells were elongate, ovalar or polygonal, with a porous structure. A special category was represented by the atypical cellular structures, that were polymorphous and sometimes mixed with cytoplasmatic particles and cellular debris. The results were analysed in the context of encouraging the use of the mammary epithelial cells in different domains of the scientific research. In conclusion, the high content of the epithelial cells in the jennet mammary secretions represent a disposable resource for the molecular study of the mammary gene expression, lactogenesis, immunity or mammary cancer.

Key words*: jennet, colostrum, cytology, differentiation, epithelial cells.*

INTRODUCTION

Despite the fact that, since the first description of the somatic cells of milk had passed almost 100 years (Donné et al., 1838; Jammes et al., 2002), nowadays we have not found any data available regarding the cytology of the jennet mammary secretions. In this context, we mention that this type of data abound in cows (Buehring et al., 1972), goats (Tateyama et al., 1988) or sheep (Lee et al., 1981; Ognean et al., 2016) and they occasionally appear in sows (Ognean et. al., 2011; Vlasiu et al., 2013), dogs (Meyer et al., 2010) or women (Gaffney et al., 1976).

The configuration and the distribution of the cellular populations in colostrum and milk are influenced by different physiological (species, physiological status, management policy) and pathological factors (mammary infections). Regarding the origin and the functions of the various types of milk cells, the most relevant studies have approached the morphological and the physiological criteria which has served as a foundation for their classification and description. The predominant cells in the colostrum and milk are represented by the components of the immune system (lymphocytes, neutrophiles and macrophages), and their involvement in the mammary gland defense totally justify the use of NCS (total number of somatic cells) as an indicator for the evaluation of the milk and mammary gland health in bovines and other species.

Together with the leukocytes, NCS include even more types of epithelial cells, which are present in milk during lactogenesis and lacto-ejection. Nowadays, a major scientific interest it is shown for the epithelial cells population. It is also noticeable the multiplication of the research which was based on the selection of

the milk epithelial cells and their characterization on the basis of the activity state and the viability level and differentiation (Meyer et al., 2010). The purpose of this study is to describe the epithelial cells population from the jennet colostrum, in order to acquire new applicable information for the evaluation of the experimental potential of the mammary epithelial cells.

MATERIALS AND METHODS

The cyto-morphological studies were carried out on colostrum samples collected from 5 jennets, with healthy mammary glands.For the health evaluation of the mammary glands we have resorted to their clinical examination, followed up by a Contrast test (Rotaru and Ognean, 1998). The colostrum samples were harvested by manual milking, after a previous preparation of the mammary glands, based on the usual sanitation measures (washing the breast, removing the first milk jets and disinfecting the teats with sanitary alcohol). Therefore, in the first 3 days after parturition, we collected average samples , consiting of at least 3 fractions of the milking and representing 5 mL for each mammary gland . The colostrum samples were processed in stained slides by using the squash technique and Dia Quick Panoptic method and then, they were examined at the microscope and they were evaluated by using the milk cytogram technique (Rotaru and Ognean, 1998). The adjustment of this cytological method to the specific of the mammary secretion required some additional laboratory tests, besides the leucogram technique (from blood), such as: the preparation of the colostrum sediment, by centrifugating (10 minutes at 3000 g) 5 ml of colostrum sample, diluted in ratio of 1:4 with physiological serum; the removal of the grease and the preparation of the smears from the obtained sediment ; degreasing the smears, by dipping in xylene or methanol.

Some adjustments made to the specific of the milk secretion also required the procedure used for registering and analysing the data, consisting of the usage of the physiological criteria for the differentiation of the cellular subpopulations and the atypical nuclear cellular structures present in the mammary secretions.

In order to identify and classify the cells types we took into consideration the following morphological criteria: the aspect of the nucleus (shape, volume, segmmentation level); the distribution of the chromatic material; the presence of the nucleolus or nuclear corpuscle. Additional physiological assessments were made concerning the features of the cytoplasm and the evolution of the activity, based on the evaluation of the nucleus/cytoplasm ratio, respectively the volume and the cytoplasmatic tinctoriality. In the cellular configuration we have also observed the cellular conglomerates, that have

resulted from the intercellular agglutinations or grease, under the form of microconglomerates. The survey of the registered data has mainly pursued the morpho-functional description of the epithelial cells types and the documentary regarding the usage of these epithelial cells in various domains of scientific research.

RESULTS AND DISCUSSIONS

Concerning the relevance of the squash technique and the Dia-Quik-Panoptic coloration, we consider that they have secured a good display and tinctoriality of the smears which were made from the colostrum; moreover,it has sustained their usage for the morphological exam of the colostrum physiological and pathological secretions, with the condition of degreasing them previously. The cellular content of the first colostrum has emphasized an extremely increased number and a high level of heterogeneous cellular populations. The overall configuration of the cellular population has revealed different types of epithelial cells, that come from lactiferous structures, respectively leukocytes (neutrophils, lymphocytes, macrophages), which come from general circulation. Other distinctive entities, with a high morphological and functional diversity have been discovered during the synthesis process and milk ejection, such as desquamated epithelial cells (Figure 1). In the following we have described and analysed the distinctive activity of the epithelial cells and the morphological criteria that has emphasized their features, respectively the differentiation of the cytoplasmic particles and the atypical cellular structures.

The obtained data underlined that the epithelial cells population, which has been identified in

the jennet colostrum, was represented by the alveolar, squamous and columnar epithelial cells.

The alveolar epithelial cells have morphologically one or two spherical nuclei, surrounded by a high volume of basophilic cytoplasm, with a circular or foamy aspect.

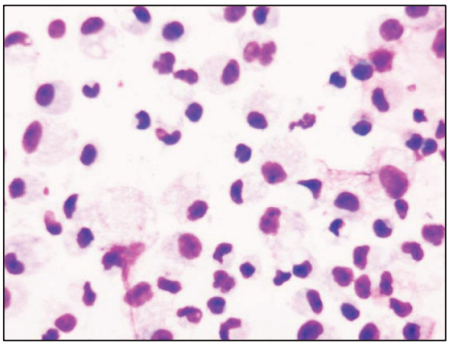

Figure 1. The overview configuration of the cellular population present in the jennet colostrum, including the alveolar cells which prevail among the other epithelial cells and different types of leukocytes (Dia Quick Panoptic Col.; x 100)

These alveolar cellular entities have different secretory activity states and they have prevailed in the epithelial cells population. The acinar cells were often difficult to be recognized because of their various morphological appearances and activity states (Figure 2).

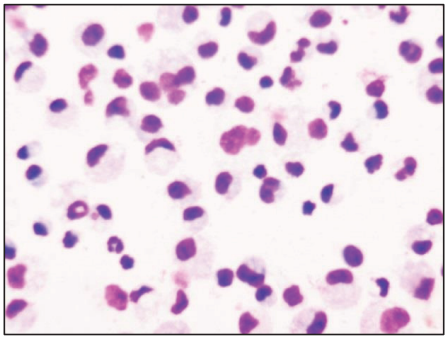

Figure 2. The predominance of the alveolar epithelial cells, in the jennet colostrum sediment, characterized by one or two spherical nuclei, surrounded by a poor basophilic cytoplasm, with a foamy aspect and grease vacuoles (Dia-Quik-Panoptic Col.; x 100)

The squamous epithelial cells have rarely been found, and their identification firstly required a differentiation from the atypical cellular structures. The main morphophisiolo-gical elements that are useful for the distinction of these cellular entities are the predominant polygonal cytoplasm and a small, punctiform and pyknotic nucleus (Figure 3).

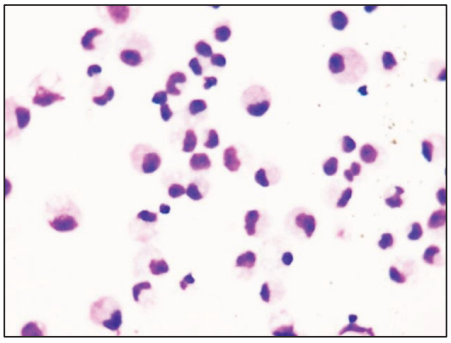

Figure 3. The presence of the squamous epithelial cells in the jennet colostrum, characterized by the polygonal cytoplasm and punctiform nucleus (Dia-Quik-Panoptic Col.; x 100)

The columnar epithelial cells were more frequent than the squamous ones. They are elongate, oval or polygonal, with a polygonal nucleus and a foamy structure (Figure 4).

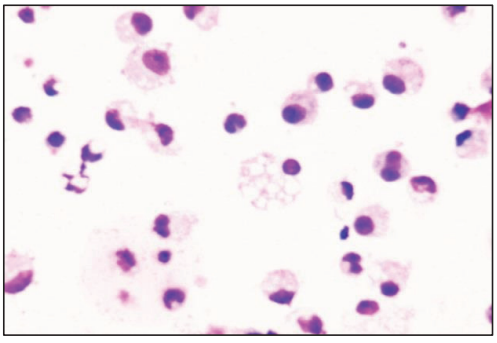

Figure 4. The presence of the columnar epithelial cells in the jennet colostrum, which are elongate,oval or polygonal, with an oval nucleus and a foamy structure (Dia-Quik-Panoptic Col.; x 100)

We consider that these epithelial cells often come from the epithelium of the mammary gland cistern and the galactophorous ducts.

The atypical cellular structures were emphasized such as polymorphic structures , which were frequently mixed with the cytoplasmic particles or anucleate cellular debris. They have a polymorphic and noticeable nucleus, surrounded by atypical morphological elements, more or less affected by the necrobiosis or apoptosis.

The mammary epithelial cells are present in

milk due to the desquamation of the lactiferous structures , that are often found in goats and other species with a predominant apocrine secretion (Taylor-Papadimitriou et al., 1981), than in cows and other species with a predominant merocrine secretion (Taylor-Papadimitriou et al., 1977). In goats and women, the increased frequency of the epithelial cells can be associated with the multiplication of the cytoplasmic particles and cellular debris (Schalm et al., 1971). In general, the cytoplasmic particles are anucleate and contain proteins (casein micelles) and lipids, without any pathological connotation. However, some of the particles may contain nucleate fragments. The origin and the function of the epithelial cells, which are strongly vacuolized and with a foamy aspect, were reported more than one hundred years ago in colostrum and during the dry period (Donné, 1838; Boutinaud and Jammes, 2002) and they were also the subject of several investigations. Therefore, some researchers regard them as desquamated epithelial cells (Gaffney et al., 1976), and others consider that they are similar to the macrophages from blood (Jensen et al., 1975; Lee et al., 1980). The epithelial cells from milk have diferent functions, including the transmission of the maternal immuno-globulins (IgA) by colostrum, a significant process of neonates immunity. The colostral immunity is a well-known mechanism in swines (Le Jan et al., 1996), leporids (Rosato et al., 1995) and ovine (Rincheval-Arnold et al., 2002).

The analysis of the cytological configuration of the colostrum reveals the augmentation of the phagocytic activity at the debut of lactation, which is very unstable and it requires the self defense mechanism of the mammary gland.

On the other hand, an increase of the PMN leukocytes at the end of colostrum period can be correlated with the lactiferous structures intensifying contact with various microbial agents, that colonize the mammary gland by ascendant way. The investigations conducted by Rotaru and Ognean (1998) had divided the cells which come from the ovine milk into leukocytes (65%) and cells with mammary origin (35%). Moreover, the increased frequency of the PMN leukocytes is associated with the evolution of different forms of mastitis.

It is widely recognised that the atypical cellular structures, the cellular debris and the acellular structures can be found in milk in cows, goats and sheep, with an increased frequency in goats (Jandal, 1996). This is a strong argument that reveals the limiting character of the indices of the mammary gland health based mainly on the somatic cells counting (NCS). The registered statistical analysis (Gonzalo et al.,1988) are relevant for the studies conducted on large sheep batches and they have established significant correlations between the increase of NCS and several factors, such as lactation period and the number of lactations.

The percentage of the epithelial cells varies during lactation and they are more common in milk than in colostrum, in women (Gaffney et al., 1976) and swine (Le Jan et al., 1996). For instance, in sows, epithelial cells represent 20-40% in colostrum and 60-90% in milk. A considerable decrease of the epithelial cells number takes place during the mammary involution (Le Jan et al., 1996). In general, the oscillations of the epithelial cells are not directly correlated with the NCS, which is more increased in the summer, mostly because of the microbial agents activity and not because of the high temperatures (Dohoo et al., 1984).

The flow cytometric analysis showed that 26% from the cells that come from the goat milk are epithelial cells, and they contributed to the removal of the dead cells. The trypan blue exclusion test of cell viability revealed that the human epithelial cells (90%) are more viabile than the epithelial cells which are present in goats milk (40%) (Boutinaud et al., 2002; Gaffney et al., 1976; Thompson et al., 1978). The apoptotic index (TUNEL test) emphasized that only 10% of the total amount of the milk cells could be apoptotic cells, because only 30% of the epithelial cells had an apoptotic DNA pattern. It was also suggested that the anucleate cytoplasmic particles could have remained unstained , and as a result, the value of the apoptotic index is lower than the cellular death rate established by the trypan blue exclusion test.

The epithelial cells represent the major component of the mammary secretion in women and swines (Buehring et al., 1972; Le Jan, 1996; Stoker et al., 1982). The majority of the species have a milk cellular population that consits mainly of leukocytes, including lym-

phocytes, neutrophiles and macrophages. They can be found in the mammary tissue and they are the primary cells of the host defense and immunity against any microbial agent (Le Jan, 1996). The macrophages prevail in bovines and ovine (35%-79%), and PMN leukocytes are predominant in goats (Rotaru and Ognean, 1998). The lymphocytes are present in the mammary secretions and they are responsible for the immunity self-defense of the neonates , especially human and swine newborns (Bertotto et al., 1990; Jain et al., 1989; Wirt et al., 1982). Furthermore, the research carried out by Rota et al., 1993 on 100 Verata breed goats underlined a more valuable increase of the lymphocytes in colostrum than in milk , in correlation with the role of the lymphocytes in the cellular immunity transmission in neonates. The macrophages release chemical mediators when they detect pathogens and as a result, they trigger the recruitment of the PMN leukocytes to the site of infection; their percentage increases from 5% to 25% in order to intensify the phagocytosis process. These arguments highlight that the percentage of the cellular structures varies depending on the species and it is different in colostrum from milk. Thus, it is estimated that the percentage of the macrophages in bovines is about 10% - 20% in colostrum and it is predominant in milk, where it represents 70% - 80% during the middle and late lactations stages. In contrast to that, the number of PMN leukocytes is higher in colostrum (50% - 80%) and lower in milk (1%) (Lee et al., 1980). Regarding the variations between different species, the higher number of PMN in the healthy goats milk can be associated with the low incidence of clinical mastitits in goats (1%). The conducted studies have showed that the increased number of the neutrophils in goats milk play an important part in protecting the animals from the mammary infections.

All data described above have revealed that the NCS oscillations can be correlated with the health condition of the mammary gland and respectively, with the quantity and the quality of the mammary secretion. Moreover, some factors such as stress, mammary involution or the immune response to an infection of the mammary gland lead to a massive requirement of the PMN leukocytes.

The quantitative and qualitative evaluation methods of the epithelial cells from milk provide useful information concerning the integrity of the mammary epithelium, the lactation stage or the effect of the milking method. Furthermore, the primary cultures of the epithelial cells from both colostrum and milk are relevant for the research of lactogenesis or galactopoiesis, mammary immunity mechanisms, cancer or mammary infections. In addition, the RNA structure that was drawn from the milk cells was eloquent for the mammary gene expression and it was also illustrative for the molecular study of the gene expression profile and its interraction with the environment.

CONCLUSIONS

The cytogram of the colostrum sediment in jennet revealed the existence of a high percentage of the epithelial cells in the configuration of a diverse and well represented cellular population, which had also included the leukocytes (neutrophils, lymphocytes and macrophages), along with the cytoplasmic particles and the atypical cellular structures. The results of our research emphasized the presence of an increased amount of the epithelial cells in the jennet mammary secretions, that can easily represent an available resource of epithelial cells that are necessary for the mammary gland gene expression profile, lactogenesis, immunity or breast cancer investigations.

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