SOMATIC CELLS AND MILK HEALTH

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Abstract: The quality of milk as an aliment (functional aliment) and finding the modifications that come along with mamitis as quickly as possible, but also other udder diseases that produce udder dysfunctions, was and remains a major problem of the medical, medical – veterinary and other domains’ research. This supposes elaborating some high sensibility tests, which, through the investigation of a small quantity of milk and in little time, would offer cert information regarding its health, respectively the possibility of early diagnosis in cases of mamitis or other abnormal udder states. After signaling the somatic cells in milk with almost a century before, they were and still are considered among the milk’s small components. Their importance for the udder health and therefore of milk milk health was revealed later, but is at present among the hygienic quality indicators of public consumption destined milk. The cytomorphologic study on the lactosediment stained May Grunwald Giemsa and somatic cell count by the ISO-13366-1 method made on raw goat and sheep milk, collected during 3 lactations, permitted us to establish with enough precision the hygienic quality (health) of it. The somatic cell count is highly superior in these species compared to cow, in the majority of cases crossing the limit imposed by the European Community. After systemizing and analyzing the gathered data in function of the somatic cell count, lactating period and the cytomorphologic aspects, the diagnosis of mamitis and identification of germ carrier animals. The cytological configuration of lactosediment smears made possible differentiating the following milk categories: healthy, normal milk, milked and presented in hygienic conditions; microbiologically intensely polluted milk; milk from animals which are at the end of the lactation period or mammary pause and also milk from animals with chronic or subacute mastitis, clinically unexpressed, with germ carrier and eliminating animals.

INTRODUCTION

Somatic cell count is a base method in the national and European milk quality control. The European Community established the legal limit for the cow milk, but without making any refers to the sheep and goat one, in the EEC/92/46 (1992) Directive. This situation was temporary, because the UE suggested establishing in the future a limit for the somatic cell count (SCC) also for the small ruminants’ milk.

In the USA, the legal limit for the SCC in the case of public consumption milk was 1,000,000 cells/ml, for the sheep milk 750,000 cells/ml, but breeders often can’t respect these (Droke et al., 1993). At the International Somatic Cells and Milk of Small Ruminants Symposium (Bella, Italy, 1993), it was suggested to the authorities of the UE at maximum limit of SCC for the tank goat and sheep milk, to be 1,500,000 cells/ml. (Barbosa et al., 1994).

Several researches in the domain tried to establish positive correlations between the total cell count and a certain type of mammary infection, and moreover, between the total cell number and the total germ count in milk. Although it was managed establishing some partial correlations between the SCC and the m.a.TGN, we consider that, to appreciate the hygienic quality of milk is necessary to complete the values of the SCC with lactocytogram, microbiologic, toxicological and physico-chemical data.
Although the sediment examination is part of the old investigation methods regarding milk integrity, the cytological examination puts this method in a new and superior form. The purpose of the cytological examination is putting a diagnosis rapidly, and in some cases a definitive one, without needing further analysis. Putting a diagnosis in a pathologic process of the mammary gland is based on the individual study of cells and the whole cell population from a milk volume.

MATERIAL AND METHOD

To test the cytological examination value in monitoring the udder health, raw milk samples were collected from an ovine and goat population, breaded in traditional system. The study was extended on three lactations – 2003, 2004, 2005, and the samples were tested for SCC through ISO-13366-1 method, the results being correlated to the cytological examination on May Grunwald Giemsa (MGG) stained smears. In some cases, to diagnose the etiological factor of the mycoplasmic mammitis, Wroblenski staining was used also.

RESULT AND DISCUSSION

The structure and proportion of the cell populations, differentiated through microscopic examination of milk sediment presented characteristic variations to the physiological state of the mammary gland, SCC varying also with the lactation phase. The highest values were obtained at the middle and end of lactation, for the goat as well as for the sheep milk.

In the MGG cytograms a sanguine origin cell population (unsomatic) was found grouping PMN cells, monocytes and lymphocytes, respectively a predominantly somatic origin cell population, including macrophages, lactocytes, epiteliocytes and other atypical cells.

The classical counting methods, based on the microscopic examination, but also the automatised ones (electronic counting), have the disadvantage that can’t establish – from the cytological point of view, which cellular component of the TCC determined at a particular moment, decreases or increases and, because of that one can’t establish with certainty the health state of the udder and the hygienic quality of milk.

At middle lactation, when the SCC raises normally, we found very high values for some milk samples, due to the apocrin type of secretion with the elimination of the nucleated or unnucleated apical pole of the cell, thereby with the presence of numerous nuclear and lactocyte debris, without the presence of any kind of inflammation (growth without an inflammatory substrate). The predominance of these cells in the milk smears with the significant modification of the TCC (total cell count) but without a cellular reaction characteristic to an inflammatory process, remains a curiosity, but also an aspect that remains to be elucidated. It’s possible however, that due to the secretion type and milk elimination in these species, and also to the period of lactation, a big quantity of apoptotic corpuscles to be eliminated – corpuscles cited by some authors, that are nothing else than lactocytes along with the apical pole of the cell (Fig. 1). The presence of nucleated or unnucleated cytoplasmatic particles of different dimensions in the goat milk (Schalm, 1971; Roganisky, 1971) mask and make very difficult interpreting the leukocyte response to infection, so that differentiating the cells between them would be of interest in clinically unexpressed mastitis diagnosis. The identification methods of leukocytes are based usually on nuclei identification.

Without minimalising the importance of TCC determination from milk, including electronic methods, the cytological study of milk, on milk smears as being but mostly on
lactosediment, can reveal more details related to the measures that should be taken when milk enters public consumption.

The significant increase of epithelial cells was observed by our team in the cases of catarrhal-desquamate or catarrhal-purulent mastitis. The cell population presents itself with a very high number of cells, disposed in groups, stuck in casein precipitates, constituting micro conglomerates of pus. There is an intense desquamation of the mammary epithelium, at all levels: mammary acins, galactophoric channels, mammary cistern, papillary channel, the cells being in necrosis and necrobiosys (Fig. 2).

![Fig. 1 - Normal, healthy goat milk citosediment, rare leukocytes and epithelial cell debris (MGG staining, x1000)](image1)

![Fig. 2 - Sheep milk citosediment, catarrhal – desquamative mastitis, high number of PMN, numerous macrophagus, pus conglomerates (MGG staining, x1000)](image2)

The most heterogenic and variable cell population from numeric and morphological point of view, proved to be the one of filtration cells, came through diapedesis in the mammary channel, and from there, in milk.

In the milk obtained from healthy udders, the lactocyto gram recorded constantly a small number of PMN cells, represented by isolated cellular elements, with lobed nuclei, well individualized lobular segments, hyperchromatic, amnofilic cytoplasm, rare phagocytated granular formations. The PMN cells find themselves along with epithelial ones, in very advanced stages of necrosis and necrobiosis, stuck into casein precipitate, mostly at the end of lactation. The PMN nucleus is intensely segmented, with the Arneth index to the left, there are cells with 4-5 or even 6 nuclei (Fig. 3). Taking into consideration the segmentation level of the nuclei, it can be said that in normal milk, old cells are predominant, in apoptosis.

In inflammatory processes, even in the clinically unexpressed ones, the morphological characteristics of PMN cells modify themselves. The number of neutrophile PMN is evidently raising, and their disposal tends to constitute small groups around the activated macrophages, or beaches through agglutination on fibrin exudate and coagulated casein. In the incipient stages of mastitis, PMN are predominant, in different evolution phases, activated for the germ and epithelial cells’ phagocytosis which are in necrosis or necrobiosis, kept into the precipitated casein coagula (Fig. 4). In this way an intense mammary epithelium desquamation takes place, with an increase in the number of cells which initiate the phagocytosis, during the primary cell response to stop the infection.
In comparison with the morphological particularities of eosinophilic PMN cells from blood, a smaller cell volume can be observed with an intensely basophilic nucleus. In the incipient stages of mastitis, when the cell population is constituted mostly of PMN cells, it is interesting that along with neutrophiles appear eosinophiles, probably due to massive mammary epithelium destruction.

Regarding the milk lymphocyte population, these were rarely observable in the lactocytosediment. Recognizing them is easy, due to the compact nucleus, hiperchromatic, round and with basophilic cytoplasm ring shaped. In the pathologic milk, the number of lymphocytes raises significantly, and morphologically, a cytoplasm tumefiation and porrose nucleus structure can be observed. A cytoplasm vacuolization can be noticed and a picnotic nucleus state, elements that plead for apoptosis. In contrast with PMN, lymphocytes don’t tend to aggregate with other cell categories from milk.

A big diversity and predomination in the cell population is represented by macrophage monocytes, which proved to be a mobile monocytic-macrophage system of the lactipher structures, present in the normal as well as in pathologic milk.

The non-activated macrophages and the activating ones are present in the normal mammary secretion. The nucleus is well individualized, kidney shaped, with clear, homogen, slightly basophilic cytoplasm. During activation, the nucleus-cytoplasm proportion modifies, the nucleus becomes condensed, hyperchromatic, round and the cytoplasm vacuolated due to the effect of the phagocytic function and pagolisosome formation, with the aspect of basophilic corpuscular structures, around which a clear ring can be noticed.

The activated macrophages are present mostly in the situations when in the mammary tissue a pathological process is in evolution, irritation or inflammation, whatever it is or not clinically expressed. The nucleus/cytoplasm proportion is in favor of cytoplasm, peripherc nucleus, kidney shaped, sometimes lobed, generalized vacuolization of cytoplasm, of variable dimensions, with foam aspect. In this case a hyperactivation of these cells can be taken into discussion, with the formation of phagosomes and phagolisosomes. The development process and formation of phagosomes and phagolisosomes becomes predominant, and the granular and foamy aspect of the cytoplasm, makes these cells easily recognizable during the cytomorphologic examination. The hyperactivated monocyte-macrophages are distinct, their number and grouping is in function of the inflammatory phase, varying from isolate elements to 2-3 cell groups, between which stable cytoplasmatic bridges form (Fig. 5).
In the case of more advanced infections, the predominant cell population is represented by that of activated macrophages, with intense phagocytosis of germs, but also of cell debris formed through the intense epithelial desquamation and PMN cells’ destruction.

A particular feature of end of lactation milk is the presence of a big monocyte-macrophagus number, but which has an own-structure phagocytic activity, transforming them in lipophages. The adherence process is very intense mostly for the milk spherules, case in which it can be noticed an adherence till in globing, situation which justifies their name of lipophages (Fig. 6).

The activation state, the hyperactivation, adherence and in globing ones reflect, in our opinion, the animal health, the mammary tissue health and by these, the hygienic quality of milk.

The lactosediment examination revealed that normal milk, retention milk, the microbiologically polluted one, confirms the pathological processes, being diagnosed in carrier and germ eliminating animals, as well as in different forms and mastitis types, in different evolution stages, in the entire animal group or not.

CONCLUSIONS

The cytomorphological researches, finalized through the counting method of these cells, led to the convincing that there is a permanent cell population in milk, of multiple origin (tissue and circulation), which morphologically differentiates and numerically varies, in function of several factors among which mammary gland inflammation on the first place.

Therefore, milk cell count, sustained by the cytomorphological study constitutes a sure monitorising method of the milk’s hygienic quality, at individual but also at group level.

The cytological smears from milk, along with the possibility of diagnosis, can be a perfect aid to offer some elements regarding the quality of milk, for example:

- a cytological smear from milk as it is but mostly from sediment, can reveal the pollution and contamination degree (microbiological hazard);
- a cytological smear can offer as well information on the contamination sources, with physical and/or microbiological hazards that milk is exposed to;
- the cytological smears can differentiate the origin of microbiological contamination from milk, if this is extra- or intra-mammary.

Fig. 5 – Goat milk citosediment, pus mastitis, numerous activated macrophagus monocytes (MGG staining, x1000)

Fig. 6 - Goat milk citosediment, retention milk, a hiperactivated macrophagus monocyte with an epithelial cell adhered (MGG staining, x1000)
Knowing the normal and pathologic aspects of the somatic cells from milk, through cytological monitorising, prevents and eliminates the biological hazard regarding this product, from the primary processing.

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