LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XL, 2007, TIMIŞOARA

ASPECTS CONCERNING MORPHOPHSIOLOGY OF CELL POPULATION FROM SMALL RUMINANTS' MILK

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Summary

The model of the milk cytogram on slides with degreased milk sediment was used in order to investigate cell population from sheep and goat milk secretion. The differentiation was performed by morphological criteria and activity stages of typical cell structures.The milk cytogram of small ruminants was characterized by increased macrophage freqvency (45.6 – 48%) and epitherial cells (7.1 – 8.3%) in colostrums and PMN leukocytes in milk (26.5 – 47.9%). The high level of lymphocytes (26.4 – 32.5%) represented a particularity for sheep milk secretions, amd abundance of atypical cells and remainders for goats.

The research concerning the goat and sheep milk cytology has been developed beginning whith 1990, so few data concerning milk cytology tests, permoed in order to monitories mammary health in this species, are available.

The data concerning cytological structure of milk reveal the high frequency of atypical cells, cell remainders and enucleate structures. These are reasons, whitch plead for the use of milk cytogram in diagnosis of mamita (Ognean, 2002). The cytological investigations are seldom performed in sheep milk, and data often concern comparison whith cow or goat milk.

Materials and methods

The cytological investigation, based on milk cytogram model, was performed on colostrums and milk samples from common breed goats (n=24) and Merino of Cluj sheep (n=23), divided in 3 groups according to lactation stage (table 1). The samples werw processed in stained slides using MGG and Panoptic methods. Besides common aspects concerning blood leukogram, milk cytogram technique included the following particularities: *preparation of milk sediment* by centrifugation (10 minutes, 2500 rpm) of 5 ml not dilued sample, 5 ml dilued sample, 1:4 ratio whit physiological serum; *degreasing of milk sediment* by slides immersion in not dilued secretions (xylene or methanol mixed whith acetate), concentration of dilued sample in order to wash the sediment, respectively; *adaptation of cytoram structure* to specific of milk secretion including physiological criteria in order to differentiate some cell subpopulation (inactive, active and hyperactive macrophages), and also the use of conventional symbols (table 1) in order to record nucleate atypical cell structures; *correlation* of data obtained from milk cytogram whith results obtained after overall study of cell content on the slide.

Results and Discussions

Concerning cytogram techniques, dilution of milk samples, and colostrums especially, recorded better results copared to degrease of slides in organic solvent, and slides stained using MGG werw of superior quality. Good quality was also obtained when Pnoptic staining was used, whith better results whith slides obtained from milk sediment, and lower from not dilued colostrums. We recommend Pnoptic staining for any milk secretion, provided dilution of secretion in mammary pause and first colostrums, whwn morphological examination is performed. We also appreciate the rapidity of this staining, which confer pragmatic qualities to milk cytogram.

The obtained data and images represent the basis of further morpho-physiological characterization of components of cell population from sheep and goat milk.

The population of PMN leukocytes was better represented in goat milk (47.9%) compared to sheep milk (26.5%), its share being lower in colostrums of both species (31.1% and 19.5%, respectively). This population of leukocytes was dominated by neutrophiles (80.7% - 93.%), whit few eozinophiles (3.7% - 12.9%), and very few basophiles (1.7% - 7%). Morphologically, mature cells whith multi-segmented nucleus surrounded or not by cytoplasm were seldom, but constant encountered.

The lymphocyte population was better represented compared to PMN cells in colostrums and sheep milk (26.4% - and 32.5% respectively), and lower in goat milk secretions (8.2% in milk, and 13.3 in colostrums). The small cells often represented only by nucleus predominanted in lymphocyte population. A lower frequency was reported for middle lymphocytes with nucleus surrounded by a thin ring made up of light basophile cytoplasm.

The macrophage cell population represented the dominant component in both goat (40.8% - 48%) and sheep (36.2% - 45.6%) milk secretions. This high heterogeneity cell population was characterized considering morphological criteria and activity stages, and the following 3 categories werw differentiated: *inactive macrophages*, predominant in sheep milk (57.6%) and characterized by almost equalnucleus – cytoplasm ratio; *active macrophages* whit nucleus – cytoplasm ratio among 1:3 and 1:5, and high frequency in colostums (32.9 – 40.4%); *hyperactive macrophages* rich in cytoplasm and whit many vacuoles, characterized by specific “foamed” aspect. The nucleus, frequently round or oval and seldom not uniform was centally situated in inactive cells and exocentric in active and hyperactive cells. Some hyperactive machrophages were lipophage, due to intra-cytoplasm fat spheres. Our data reveal the intensifying of phagocyte activity in colostrums, which can be correlated to high share of hyperactive macrophages during the beginning of lactation (18.2 – 27.2%). We also noticed he modified aspect of some hyperactive phagocytes affected by necrobiosis or apoptosis, which made difficult

or even impossible their identification. Such processes may be involved in formation of colostrums structures (*Rotaru and Ognean,* 1998).

Table 1.
The average values of milk cytogram of goats (groups 1 and 2) and sheep (groups 3 and 4) dusting colostums secretion and lactation.

Types of cells	Group 1 (n=11)		Group 2 (n=13)		Group 3 (n=11)		Group 4 (n=12)	
PMN leukocytes	114	31.14	162	47.92	54	19.56	57	26.51
Neutrophyles	*104*	*91.22*	*152*	*93.82*	*45*	*83.33*	*46*	*80.70*
Eozinophiles	*8*	*7.01*	*6*	*3.70*	*7*	*12.96*	*7*	*12.28*
Basophiles	*2*	*1.75*	*4*	*2.76*	*2*	*3.7*	*4*	*7.01*
Lymphocytes	50	13.66	28	8.28	73	26.46	70	32.55
Macrophages	176	48.09	138	40.82	126	45.65	78	36.27
Inactive	*70*	*39.77*	*54*	*39.13*	*52*	*41.26*	*45*	*57.69*
Hyperactive	*48*	*27.27*	*36*	*26.08*	*23*	*18.25*	*8*	*10.25*
Epitherial cells	26	7.10	10	2.97	23	8.33	10	4.65
Total	366	100	338	100	276	100	215	100
Atypical cell structures *	+++ +		++		++		+	

*- nucleate cell structures in apoptosis or necrobiosis, sometimes represented only by nucleus: + = 1-15 elements/microscopic field; ++ = 16-30 elements; +++ = 31-45 elements; ++++ > 45 elements.

The epitherial cells presented morphological traits correspondent to desquamated epithelium: plane, cylindrical, alveolar.

Plane epiteliocytes characterized by polygonal cytoplasm mass and punctiform nuceus werw noticed only in few colostrums samples. Some elongated cells in oval or polygonal shape and porous structures, which can have origin in cysternal or great galactophorus channels epithelium, were identified. Majority of slides emphasized cells whith spherical nucleus surrounded by large basophile cytoplasm ring or foamed in shape, which can have origin in alveolar epithelium, representing lactocytes in different stages of activity.

The atypical cells structures werw emphazed as polymorph structures predominant in goat colostrums (++++), which even had nucleus could not be framed in alredy known cell categories. As presented in table 1, frequency of different cell types recorded more or less important variations in milk secretions of both ruminant species. We noticed the higher share of PMN leukocytes in goat milk (47.9%) and lymphocytes in sheep milk (32,5%). The predominant active and hyperactive macrophages represented almost majority cell population both in goat (48%) and sheep (45.6%) colostrums and epithelial cells were more frequent in colostrums (8.3 – 7.1%) caompared to milk (2.9 – 4.6%).

The data interpretation reveals the intensification of phagocyte activity during colostrums secretion, indicating the enhancement of "self cleaning" processes in mammary gland in the beginning of lactation (*Zeng*, 1996). The light increase of PMN leukocytes frequency in the end of colostrums secretion ang during lactation can be exp[lained by intensification of the contact between milk structures and different microbial agents, wich colonize mammary gland by ascendant way (Zeng and Escobar, 1996). This evolution shows that PMN leukocytes represent the milk cells that determine variations of all other cell types, in the mean time reflecting heath status of mammary gland (*Lee et Al,* 1981). Such an explanation was also advanced by *Guyonet at al* (1986), which divided cells from goat milk in leukocytes (65%) and cells of mammary origin (35%) the increase of leukocytes (mainly represented by PMN) frequency being correlated to evolution of different mamites, when their proportion can rich 80% (*Jandal,* 1996). Similar to our results, data obtained by *Rota* et al (1993) from 100 Verata goats, revealed a larger number of lymphocytes in colostrums compared to milk, which was attributed to their implication in transmission of cell immunity to offspring. The above mentioned authors grouped in the same place the epitherial cells and other cell types and attribute them higher frequency as compared to our results concerning recorded epitheliocytes (2.9 – 8.33%).

Conclusions

The obtaining and degreasing of milk sediment deterined the quality of slides, representing specific stages for milk cytogram.

1. The average values of sheep and goat milk cytogram presented variations during lactation, represented by predominance of macrophages in colostrums (45,6% - 48%) and PMN leukocytes in milk (26.5% - 47.9%).
2. The differences concerning the representation of different cell types in milk cytogram of both species werw revealed by increase of PMN leukocytes frequency in goat milk (31.1% - 47.9%)and lymphocytes in sheep milk (26.4% - 32.5%).
3. The differentiation of macropfages by activity stages reveald the predominance of active (32.9% - 40.4%) and hyperactive cells (18.2% - 27.2%) in colostrums and intensyfing of phagocitosis in the beginning of lactation.
4. Identified by morphological criteria and separately classified, epitherial celle were more frequent in colostrums (7.1% - 8.3%) compared to milk (2.9% - 4.6%).
5. The atypical cell structures exclusively represented by necrobiosis or apoptosis nucleate structures were predominant in goat colostrums (++++).

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