Somatic (CSS) and differential cell count (DCC) during a lactation period in ass’ milk

Daniela Beghelli1, Annamaria Roscini1, Andrea Valiani2, Silvia Vincenzetti3, Clarita Cavallucci1, Paolo Polidori1

1Dipartimento di Scienze Ambientali. Università di Camerino, Italy
2IZUM. Perugia, Italy
3Dipartimento di Scienze Morfologiche e Biochimiche Comparete. Università di Camerino, Italy.

Corresponding author: Daniela Beghelli. Dipartimento di Scienze Ambientali, Sezione di Produzioni Animali. Facoltà di Medicina Veterinaria, Università di Camerino. Via Circonvallazione 93/95, 62024 Matelica (MC), Italy - Tel. +39 0737 40 34 38 - Fax: +39 0737 40 34 02 - Email: daniela.beghelli@unicam.it

ABSTRACT - Hypoallergenic properties of ass’s milk protein fractions have been recently confirmed, allowing ass’s milk to be considered as a valid substitute of the available hypoallergenic infant formulas. The objective of this study was to give a further contribution to the knowledge of ass’s milk safety and quality characteristics. A new procedure has been developed with a cytospin centrifuge in differential counts of milk somatic cells. Somatic cells count (SCC), differential somatic cells count (DCC) and cultural examinations have been carried out in 62 milk samples collected from 11 asses at three different stages of lactation. Four major cells populations had been identified in ass’s milk too: lymphocytes (Ly), monocytes/macrophages (MA), polymorphonuclear neutrophils (PMNL), and epithelial cells (CE). The patterns of these cells have been discussed in comparison with cells found in dairy cows and ewes milk. In conclusion, a reproducible standard procedure has been developed to determine cell count of ass’s milk.

Key Words: Ass’s milk, SCC, DCC, Cytospin.

Introduction - Ass’s milk continues to elicit a big interest in the Scientific Community for its nutritional and nutraceutical characteristics. The beneficial ass’s milk effect on skin had been know since Cleopatra period; nowadays, besides its use in the cosmetic industry, it seems to be the best substitute of human milk in infant nutrition (Polidori, 1994; Salimei et al., 2006; Agostino e Palmesi, 2007). The ass’s milk is rich in lactose (the taste of ass’s milk resembles the breast milk), lysozime, ω-3 and ω-6 polyunsaturated fatty acids (Chiofalo et al., 2004); the ash residual is similar to the human milk one, whereas the proteins’ profile is adequate for a correct development of the infants’ digestive tract. Considering that ass’s milk is consumed by human infants (in particular, those with multiple food allergies or Cow Milk Protein Allergy) (Iacono, 2002) and ageing people (Conte, 2007) it is necessary to deep the knowledge not only on the ass’ milk quality but also on safety. The somatic cell count (SCC) represents a routine system to evaluate the sanitary status of the mammary gland and the quality of milk (O’Brien et al., 1999; Fthenakis et al., 1991; Sordillo et al., 1997; Saad & Östensson, 1990; Fruganti et al., 1983). During an infection/inflammation of the mammary gland (mastitis), in fact, there is a remarkable trafficking of leukocytes from the blood/lymphatic vessels to the gland and this is supported from an increase of milk SCC and a modified Differential Cell Count (DCC) (Morgante et al., 1994). Although some individual/physiological factors could affect the SCC (Östensson, 1993); more often the increase of SCC and the modification of DCC should be assigned to the presence of mastitis (Paape et al., 1991; Saad & Östensson, 1990). In this study the health status of the ass’ mammary gland was evaluated during the lactation period, monitoring SCC and DCC modifications in the milk.
Material and methods - The trial was carried out from October 2007 until July 2008 on 11 pluriparous jennets (n.6 Martina Franca, n.5 Amiantina), randomly selected. Foals were physically separated from the dams 3 hours before the milking. Sterile milk samples were collected from each emi-mammary gland at the first month of lactation (Period 1), at middle lactation (Period 2) and nearly at the end of the lactation period (about 5-6 months; Period 3).

Since in literature were not available protocols to evaluate the DCC in ass’ milk, in this study a new procedure for this kind of milk had been set up.

Firstly, the mammary glands were cleaned with a mixture of alcohol-ether (1:1) and then they were hand-milked, after the discharge of the first three ejections. Milk samples were collected into two sterile tubes/emi-mammary gland (Falcon 50 ml):

one milk sample was used for the microbiological tests (milk was firstly disseminated in Blood Agar plates, then, in Mac Conkey and /or Tripticase Soy Agar) and SCC (by a fluoro-opto-elettronic method: Fossomatic 5000 – Foss-electric);

the second milk sample, collected with an our mixture of anticoagulants (4 ml of 3,2 acid citrate dextrose and 180 μl of EDTA, 1 M solution at pH 8.0) was used for the DCC.

Briefly, the milk samples were spun at 1600 rpm for 25 minutes at 5-6°C, then the pellets were twice washed in RPMI 1640 (ibi International, Milano), and spin again at 1200 rpm, 15 min., 15°C. The final pellets were resuspended in 1000 μl of RPMI 1640, divided in two aliquots and dispensed in a cytospin (600 rpm for 10min.) (Rotofix 32, Hettich) to prepare some slides for the optical microscope. The slides were stained with May-Grunwald Giemsa stains (Fruganti et al., 1983). By counting (expressed as %) the first 100 cells of the smears (at a 1000X magnification) and the following types of cells were identified: neutrophils (PMNL), lymphocytes (Ly), monocytes/macrophages (MA), eosinophils (EO), and epithelial cells (EC). The obtained data were processed to the analysis of variance (ANOVA, SPSS).

Results and conclusions - Microbiological and SCC results seem to testify a good health status of the investigated animals. Mean values of the SCC/ml were under the 100.000 cells/ml (Table 1), although with a wide standard deviation that could be explained by the low number of animals. The lactation phase did not significantly influence the SCC, although the highest SCC were registered at the beginning and end of the lactation (Table 1), according to data reported for other dairy species (Zeconci & Smith, 2000).

| PERIOD | N. of samples | SCC/ml | Lymphocytes % | Macrophages % | PMNL % | CE % |
|--------|---------------|--------|----------------|---------------|--------|------|
| 1      | Mean          | 20     | 43.400         | 10            | 21.5   | 18.0 | 54.6 |
|        | ds            |        | 46.095         | 5.3           | 9.6    | 8.6  | 20.0 |
| 2      | Mean          | 22     | 14.929         | 17            | 19.4   | 46.6 | 19.7 |
|        | ds            |        | 14.736         | 12            | 8.2    | 19.6 | 12.4 |
| 3      | Mean          | 20     | 25.875         | 12            | 12.5   | 54.0 | 9.5  |
|        | ds            |        | 32.839         | 7             | 5.7    | 16.5 | 8.5  |
| P      | ns            |        | ns             | ns            | 0.05   | ns   | ns   |

The DCC (expressed as %) (Table 1) showed, some trends for MA (P<0.05), PMNL (P: n.s.) and EC (P: n.s.) during the lactation. The number of PMNLs increased, becoming the largest ones. Numerically, MA are the second ass’ milk cells type and they showed, as the ECs do, an inverse trend during lactation. The percentage value of Ly remained substantially constant during the lactation. The ass’milk somatic cells, expressed in absolute terms, resembled the trend of cells expressed in percentages, with the exception of the PMNLs that showed their minimum values at period 2 (Graphic 1). The DCC of the other dairy spe-
cies, evaluated during a lactation period in an healthy mammary gland, shows a prevalence of MA cells (Concha, 1986; Paape et al., 1991; Sordillo et al., 1997; Zecconi & Smith, 2000). At present, these are the only data on ass’milk DCCs. The PMNLs predominant cells in the milk secretion, during an entire lactation, seems to be an unique characteristic of equine physiology, when compared to the dairy ruminants.

Graphica. DDC, expressed in absolute value (cells/ml), in three at different periods.

The research was supported by Ministry of Agricultural (Coordinator: Prof. Polidori P.).

REFERENCES - Agostino, R., Palmesi, F., 2007. Allattamento materno e latte d’asina. Atti del II° Convegno nazionale sul latte di asina: “Latte d’asina: perché?” Roma, 22 Marzo 2007. Chiofalo, B., Azzara, V., Ventincine, L., Piccolo, D., Chiofalo, L., 2004. Variations of fatty acids in Ragusana Ass’s milk. 55th A. Meet. of the EAAP, Bled, Slovenia. Concha, C., 1986. Cells types and their immunological functions in bovine mammary tissues and secretions. A review of the literature. Nor. Vet. Med., 8: 252-272. Conte, F., 2007. Linee guida per il controllo igienico-sanitario del latte d’asia. Atti del II° Conv. Naz. sul latte d’asina: Latte d’asina: Perché?. Roma, 22 Marzo 2007. Fruganti, G., Ranucci, S., Valente, C., 1983. Sul valore diagnostico di alcune prove di laboratorio nelle mastiti della pecora. Clin Vet., 106: 145-155. Fthenakis, G. C., El-Masannat, E. T. S., Booth, J. M., Jones, E. T., 1991. Somatic cell counts of ewes’s milk. British Vet. J. 147, 6. Iacono, G., Carrocio, A., Cavataio, F., Montalto, G., Soresi, M., Balsamo, V., 1992. Use of ass’milk in multiple food allergy. J. of Ped. Gastr. and Nutr. 14, 177–181. Morgante, M., Ranucci, S., Casoli, C., 1994. Caratteristiche citologiche del secreto mammario di pecore pluripare in lattazione. Small Rum. Res. 3: 55-56. O’Brien, B., Fitzpatrick, C., Meaney, W. J., Joyce, P., 1999. Relationship between somatic cell count and neutrophils in milk. Irish J. Agric. Food Res. 38, 288-296. Östensson, K., 1993. Variations during lactation in total and differential leukocyte counts, N-acetyl-β-D-glucosaminidase, antitrypsin and serum albumin in foremilk and residual milk from non infected quarters in the bovine. Act. Vet. Scand. 34, 83-93. Paape, M. J., Guidry, A. J., Jain, N. C., Miller, R. H., 1991. “Leukocytic defense mechanism in the udder”. Flem. Vet. J. 62 (suppl. 1) : 95-109. Polidori, P., 1994 Il latte dietetico. Proc. of Aspetti dietetici nella produzione del latte, un alimento antico proiettato verso il futuro symposium, Torino, Italy, pp. 47–58. Saad, A. M., Östensson, K., 1990. Flow cytofluorometric studies on the alteration of leukocyte populations in blood and milk during endotoxin- induced mastitis in cows. Am. J. Vet. Res., 51: 1603-1607. Salimei, E., Funtuz, F., Polidori, P., Coppola, R., Chiofalo, B., Varisco, G., 2006. Ass’s milk: Nutritional and functional characteristics. In: 6th ILFSS Proc. Book-EAAP publication, No. 118: 93-98. Sordillo, L. M., Shafer-Weaver, K., De Rosa, D., 1997. Immunobiology of the Mammary Gland. J. Dairy Sci. 80: 1851-1865. Zecconi, A., Smith, K.L., 2000. IDF Position Paper on Ruminant mammary gland immunità. In : Symposium on Immunology of Ruminant Mammary Gland, Stresa, 11-14 June. 31-33. Zecconi, A., 2007. Le cellule somatiche nel latte influenza sanità e qualità. Linformatore agrario, 8: 66-70.