NPPC NATIONAL AGRICULTURAL AND FOOD CENTRE RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 52, 2019 (4): 160–165
© 2019 NPPC
ISSN 1337-9984
E-ISSN 1338-0095

DISTRIBUTION OF LEUCOCYTES AND EPITHELIAL CELLS IN SHEEP MILK IN RELATION TO THE SOMATIC CELL COUNT AND BACTERIAL OCCURRENCE: A PRELIMINARY STUDY

Jaromír VAŠÍČEK[1,2]*, Kristína TVAROŽKOVÁ[3], Michal UHRINČAŤ[1], Lucia MAČUHOVÁ[1], Lukáš HLEBA[2], Vladimír TANČIN[1,3]

[1]NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[2]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Nitra, Slovak Republic
[3]Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Nitra, Slovak Republic

ABSTRACT

Milk somatic cell count (SCC) is a main indicator of udder health in dairy animals. Thus, increased SCC levels are usually associated with the clinical and/or subclinical intramammary infections. SCC are mainly composed of immune cells (leukocytes) and epithelial cells. Recently, several flow cytometric approaches were used to assess the distribution of these cells in the milk of ewes. Hereby, a new combined antibody panel was designed for this purpose. Briefly, milk cells were stained with specific antibodies: CD18 (leukocytes), CD21 (B cells), CD4 (Th cells), CD8 (Tc cells), CD14 (monocytes) and CD11b (polymorphonuclear cells – PMNs). CD18 negative cells were considered as epithelial cells. Moreover, a qualitative examination of bacteria species presented in the milk was carried out using MALDI-TOF MS. Analysed milk samples were divided into 5 classes according to the SCC number as follows: <300,000 cells.ml^{-1} (SCC1), 300,000-500,000 cells.ml^{-1} (SCC2), 501,000-1,000,000 cells.ml^{-1} (SCC3), 1,001,000-2,000,000 cells.ml^{-1} (SCC4) and >2,000,000 cells.ml^{-1} (SCC5). SCC1-2 samples were considered as normal milk samples, whereas SCC3-5 as abnormal samples. Bacteriological assessment revealed that all samples in SCC3-5 class were infected mainly by *S. epidermidis* and *S. caprae*. On the other hand, SCC2 did not exhibit a pathogen infection and in SCC1 only 22 % of samples were infected. Concerning the somatic cell composition, SCC1-2 classes comprised approximately 50:50 of leukocytes and epithelial cells. The main leukocyte subsets were PMNs. However, the number of leukocytes alongside with PMNs count significantly ($P<0.05$) increased in SCC3, whereas the number of epithelial cells significantly ($P<0.05$) decreased compared to SCC1-2. Similar trend, although not significant, was observed in SCC4-5 samples. The proportion of nonviable PMNs also increased ($P<0.05$) in SCC3, however it was not markedly different in comparison to live PMNs among all SCC classes. In conclusion, described methodological approach could be effective in the more detail further research dealing with distribution of different cells of different origin (epithelial, leukocytes) in cases of subclinical mastitis caused by different mastitis pathogens.

Key words: sheep; milk; SCC; bacteria; leukocytes; flow cytometry

INTRODUCTION

Milk belongs to the basic human foodstuff. Thus, quality of milk should be thoroughly controlled before it enters the human consumption channels. The milk somatic cell count (SCC) is the basic indicator of udder health and thus milk quality, safety and technological properties of all dairy animals. Although the United States established a SCC limit for goats and sheep at 1,000,000 cells.ml^{-1}, the European Union limited the maximum SCC for cows, but not for goats and sheep (Paape *et al.*, 2007). However, Maurer and Schaeren (2007) and (Tančin *et al.*, 2017) described that SCC is highly

***Correspondence:** E-mail: jaromir.vasicek@nppc.sk
Jaromír Vašíček, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic

Received: October 10, 2019
Accepted: November 12, 2019

associated with the infection of udder halves. Therefore, there is a general agreement that the limit of 500,000 cells.ml^{-1} could be considered as an indicator of health problems in ewe's udder (Tančin *et al.*, 2016). The SCC in small ruminants contains the different cells types presented in milk, including leukocytes and epithelial cells (Souza *et al.*, 2012). Recently, it was observed that proportion of different immune cells can be measured in milk of cows (Leitner *et al.*, 2012; Li *et al.*, 2015), goats (Bagnicka *et al.*, 2011; Boulaaba *et al.*, 2011; Leitner *et al.*, 2012) and sheep (Albenzio and Caroprese, 2011; Albenzio *et al.*, 2012; Leitner *et al.*, 2012; Świderek *et al.*, 2016) by flow cytometry using different species specific antibodies.

In this study, a novel antibody panel is used for the determination of leukocytes subsets and epithelial cells in the milk of ewes and their relationship with SCC and the presence of bacteria.

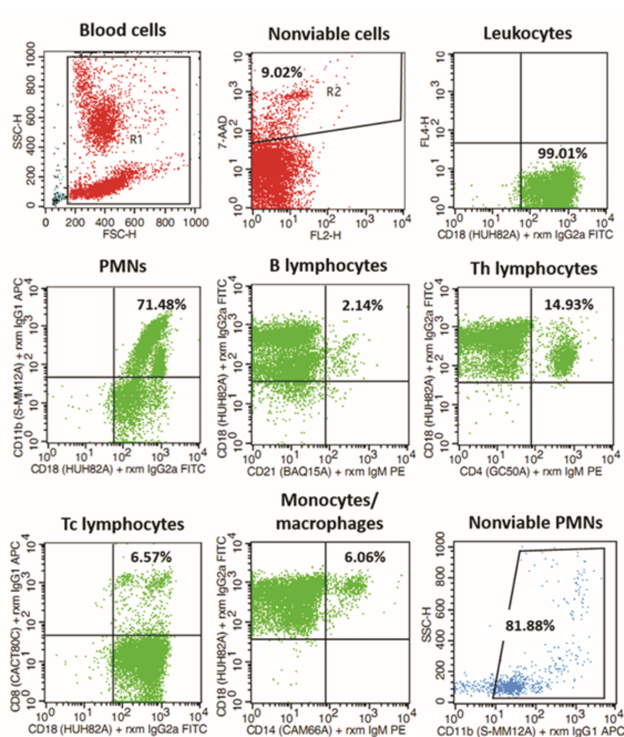
MATERIAL AND METHODS

Ten ewes of Tsigai breed from the local farm were used in the experiments. In total, 20 milk samples from each udder half were collected for bacteriological cultivation, evaluation of SCC and flow cytometry. For pathogen detection the milk samples were collected by discarding first squirts of milk and subsequently cleaning of the teat end with 70 % alcohol and milk sample from each udder halves was taken in sterile tube. The inoculum of each sample was inoculated onto blood agar (Oxoid LTD, Hamshire, UK). All plates were incubated aerobically at 37 °C and examined after 24 hours. Examination of bacteria in milk samples was performed using MALDI-TOF mass spectrometry (Bruker Daltonics, Germany). For bacterial analysis cells from a one colony of fresh culture were used for isolate to prepare samples according to the microorganism profiling ethanol-formic acid extraction procedure as recommended by the manufacturer. Samples spot was overlaid with 2 µl of matrix solution (saturated solution of α-cyano-4-hydroxy-cinnamic acid in 50 % acetonitrile with 2.5 % trifluoroacetic acid) and allowed to dry for 15 min (Bruker Daltonics GmbH, Germany). To identify microorganisms, the raw spectra obtained for each isolate were imported into BioTyper software version 2.0 (Bruker Daltonics). Contagious pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*) were classified as positive if one or more colony-forming unit (CFU) were found. Minor and environmental mastitis pathogens were classified as positive if at least five CFU were found. Samples were classified as contaminated if three and more pathogens were isolated from one milk samples and growth of contagious pathogens was not identified. Somatic cell count was analysed using a Somacount 150 (Bentley Instruments, Inc., Chaska, Minnesota, USA). Milk samples were divided into 5 classes according to the somatic cell count as described previously by Albenzio *et al.* (2012): < 300,000 cells/ml (SCC1), 300,000-500,000 cells.ml^{-1} (SCC2), 501,000-1,000,000 cells.ml^{-1} (SCC3), 1,001,000-2,000,000 cells.ml^{-1} (SCC4) and > 2,000,000 cells.ml^{-1} (SCC5). According to the above-mentioned studies, milk samples were considered as milk with low SCC (SCC1-2) and high SCC (SCC3-5).

Milk samples for flow cytometry were processed as described by Sarikaya *et al.* (2004). Briefly, 50 ml of each milk sample were centrifuged for 30 min. and 1500 x g at 4 °C. The fat layer on the top of the tubes was carefully removed and supernatant (skim milk) was discarded. The cell pellet was washed in 5 ml of ice-cold PBS (Biowest, USA) and centrifuged for 15 min. and 460 x g at 4 °C. Automated cell counter EVE^{TM} (NanoEntek, USA) was used to determine the cell concentration. Cells were then divided into prepared tubes and stained with the ovine specific primary mouse monoclonal antibodies (all from WSU, USA) according to the producer's manual in order to distinguish between leukocytes subsets and epithelial cells as follows: anti-CD18 (HUH82A, Ig2a) for all leukocytes, anti-CD21 (BAQ15A, IgM) for B lymphocytes, anti-CD4 (GC50A, IgM) for T helper lymphocytes, anti-CD8 (CACT80C, IgG1) for T cytotoxic lymphocytes, anti-CD14 (CAM66A, IgM) for monocytes/macrohages and anti-CD11b (S-MM12A, Ig1) for live and nonviable polymorphonuclear cells (PMNs, granulocytes). The specificity of the used monoclonal antibodies was proved by staining of ovine blood samples (Figure 1). Proper rat anti-mouse (anti-IgG2a, anti-IgM and anti-IgG1) fluorochrome conjugated (FITC, PE and APC, respectively) antibodies were used as secondary antibodies. Heat inactivated sheep serum was used to block the unspecific antibody binding.

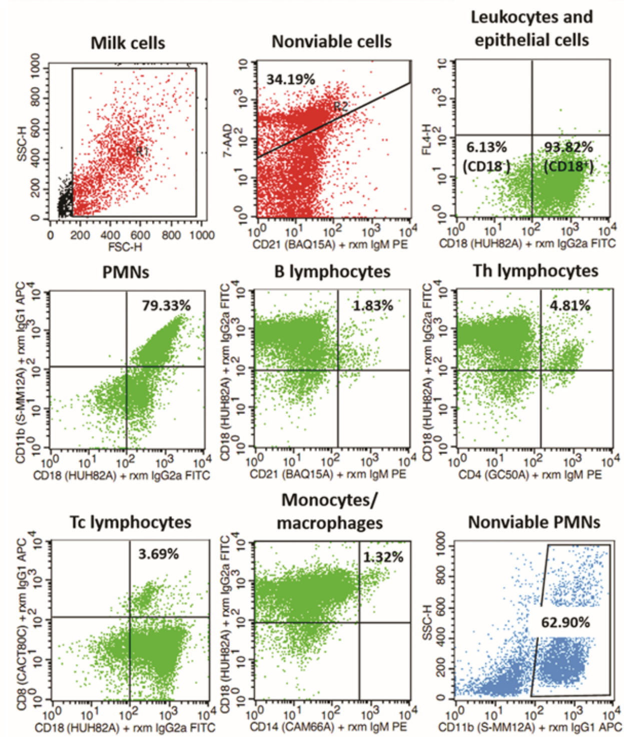
To determine the viability of analysed cells and/or to exclude the dead cells from the analysis 7-AAD (Thermo Fisher Scientific, USA) staining was used. At least 10,000 cells were analysed in each sample using flow cytometer FACSCalibur (BD Biosciences, USA). The leukocytes subsets were counted within the $CD18^+$ cells. The $CD18^-$ cells were considered as epithelial cells. The evaluation strategy is shown in Figure 2.

Obtained results were evaluated using the SigmaPlot software (Systat Software Inc., Germany) with one-way ANOVA (Holm-Sidak method) and expressed as the means ± SEM.



R1 – all leukocytes excluding cell debris and erythrocytes, R2 – all nonviable cells within R1 region, PMNs – polymorphonuclear cells, Th – T helper, Tc – T cytotoxic. Leukocytes subsets were counted within R1 region excluding the cells in R2 region. Nonviable PMNs were counted in R1 and R2 region.

Figure 1. Illustrative distribution of leukocyte subsets in ovine peripheral blood



R1 – all milk somatic cells excluding cell debris, R2 – all nonviable cells within R1 region, $CD18^+$ - leukocytes, $CD18^-$ - epithelial cells, PMNs – polymorphonuclear cells, Th – T helper, Tc – T cytotoxic. Leukocytes subsets were counted within R1 region excluding the cells in R2 region. Nonviable PMNs were counted in R1 and R2 region.

Figure 2. Illustrative distribution of leukocyte subsets and epithelial cells in milk of ewes

RESULTS AND DISCUSSION

In general, clinical mastitis resulted in low milk yield and in increased somatic cell counts (Gonzalo *et al.*, 1994; Leitner *et al.*, 2004). However, Silanikove *et al.*, (2005) also reported milk production losses caused by subclinical mastitis (SCM). The main cause of mastitis is usually an infection of different bacteria such as coagulase-negative *Staphylococcaceae* (Rupp *et al.*, 2003, Holko *et al.*, 2019). SCM and/or intramammary infection (IMI) are often indicated by the high somatic cell count. Persson *et al.* (2017) observed that a high SCC (≥400,000 – 500,000 cells.ml^{-1}) in the ewe's milk of mainly Swedish breeds was associated with IMI. In addition, Kern *et al.* (2013) also concluded that SCC might indicate an udder health problem. Our findings (Table 1) are in agreement with above mentioned reports, as the presence of bacteria increased

Table 1. Proportion of immune and epithelial cells in sheep milk with different somatic cell count

SCC class	SCC1 (n = 9)	SCC2 (n = 4)	SCC3 (n = 2)	SCC4 (n = 2)	SCC5 (n = 3)
SCC (x 10^3 cells.ml^{-1})	104 ± 17	347 ± 20	598 ± 81	1838 ± 33	3482 ± 583
Bacteriological- -positive samples (%)	22 % (contaminated – 11 %, *S. caprae* – 11 %)	N	100 % (*S. epidermidis*)	100 % (*S. caprae*)	100 % (S. epidermidis – 33%, *S. caprae* – 67%)
Leuko – CD18$^+$ (%)	49.13 ± 9.34^a	49.01 ± 7.60^a	99.46 ± 0.25^b	86.60 ± 7.23	79.40 ± 6.35
PMNs – CD11b (%)	27.15 ± 9.84^a	18.41 ± 14.9^a	98.35 ± 0.32$^{b, A}$	60.04 ± 19.29	62.66 ± 11.59
B lym – CD21$^+$ (%)	2.47 ± 1.07	4.97 ± 3.02	0.50 ± 0.25	2.26 ± 0.43	2.27 ± 0.37
Th lym – CD4$^+$ (%)	5.19 ± 2.27	10.66 ± 8.89	0.96 ± 0.71	11.56 ± 6.75	5.62 ± 2.51
Tc lym – CD8$^+$ (%)	3.61 ± 0.95	7.20 ± 5.05	0.36 ± 0.19	8.36 ± 4.69	4.88 ± 1.43
CD4$^+$/CD8$^+$ (%)	1.40 ± 0.34	1.05 ± 0.28	2.24 ± 0.77	1.35 ± 0.05	1.08 ± 0.22
Mono/Macro – CD14$^+$ (%)	3.35 ± 1.32	4.76 ± 4.48	1.86 ± 0.47	3.02 ± 1.70	2.60 ± 0.62
Epithel – CD18$^-$ (%)	50.44 ± 9.28^a	50.19 ± 8.06^a	0.48 ± 0.22^b	13.35 ± 7.22	20.30 ± 6.15
Nonviab – 7-AAD$^+$ (%)	34.28 ± 6.05	23.02 ± 5.90	7.73 ± 1.16	42.84 ± 13.70	56.24 ± 12.88
Nonviab PMNs – CD11b$^+$ (%)	30.32 ± 9.04^{a,c}	46.03 ± 9.35^a	90.23 ± 0.84$^{b, B}$	57.77 ± 5.13	79.28 ± 3.49^d

SCC – somatic cell count, SCC1: < 300,000 cells.ml^{-1}, SCC2: 300,000-500,000 cells.ml^{-1}, SCC3: 501,000-1,000,000 cells.ml^{-1}, SCC4: 1,001,000-2,000,000 cells.ml^{-1}, SCC5: >2,000,000 cells.ml^{-1}, Leuko – leukocytes, PMNs – polymorphonuclear cells (granulocytes), B lym – B lymphocytes, Th lym – T helper lymphocytes, Tc lym – T cytotoxic lymphocytes, Mono/Macro – monocytes/macrophages, Epithel – epithelial cells, Nonviab – nonviable cells, contaminated – three and more pathogens, N – no pathogen, ^{a} vs. ^{b} and ^{c} vs. ^{d} are statistical different at P < 0.05 within the same row, ^{A} vs. ^{B} are statistical different at P < 0.05 within the same column.

in milk samples with abnormal SCC (100 % in SCC3-5) in comparison to samples with normal SCC (22 % or without pathogen in SCC1 and SCC2, respectively). The most often occurring bacteria species in the presented milk samples were coagulase negative staphylococci: *S. epidermidis* and *S. caprae*. These species are commonly reported also by others (Albenzio *et al.*, 2012; Świderek *et al.*, 2016).

Milk of dairy animals such as cows normally contain somatic cells that are usually composed of leukocytes and released glandular epithelial cells (~ 50 % of both) (Shoshani *et al.*, 2000). The ratio of polymorphonuclear cells to mononuclear (lymphocytes and monocytes/macrophages) is usually ~ 1 in the case of the health animals (Dosogne *et al.*, 2003; Mehne *et al.*, 2010). Similar observation was noticed in our study as the leukocytes and epithelial cells in the normal milk samples (SCC1-2) were presented at the ratio 1:1 (Table 1).

In this preliminary study, three different flow-cytometric approaches (Albenzio *et al.*, 2012; Leitner *et al.*, 2012; Świderek *et al.*, 2016) were combined in order to design novel and complex antibody panel for the relatively rapid evaluation of somatic cell composition of the sheep milk samples. Due to this panel, a high decrease in the number of epithelial cells was found in the all abnormal SCC classes (SCC3-5), although only value in SCC3 class was significantly different (P < 0.05) in comparison to normal classes (SCC1-2). In contrast, the leukocyte counts significantly increased (P < 0.05) in SCC3 compared to SCC1-2. This value increased also in SCC4 and 5, although not significantly. Alongside with the rise of leukocyte number, the proportion of PMNs also increased significantly (P < 0.05) in SCC3 and non-significantly in SCC4-5 compared to SCC1-2. On the other hand, no significant differences were observed in the proportion of B cells, T cell subsets (CD4$^+$ and CD8$^+$) or their ratio (CD4$^+$/CD8$^+$) and macrophages among the SCC classes (Table 1). Similarly, Leitner *et al.* (2012) noticed significant increase of leukocytes, PMNs and monocytes in infected samples compared to bacteria-free milk samples with no differences between CD4$^+$ and CD8$^+$ T cells. On the contrary, significant decrease in PMNs and macrophages and increase in lymphocytes in the order from SCC1 to SCC5 class was reported by Albenzio *et al.* (2012). These authors also found significant differences in proportion of T cells (both CD4$^+$ and CD8$^+$) and their ratio CD4$^+$/CD8$^+$

among the observed classes. Moreover, they suggested the nonviable PMNs for an indicator of inflammatory status of the ewe udder since their proportion significantly increased from SCC1 to SCC5, whereas number of live PMNs decreased. Our study confirmed the growing tendency of nonviable PMNs in association with increasing SCC (Table 1). However, we did not notice significant changes in live and nonviable PMNs among the studied SCC classes, except for the class SCC3 (98 % vs. 90 %, respectively). We also did not find significant differences in the number of total dead cells within the samples. Thus, the usefulness of this indicator in contrast to the live PMNs is questionable; even take into account the possible unspecific binding of the antibodies to dead cells.

In fact, IMI causes a rise in the number of milk somatic cells during lactation, due to the infiltration of leukocytes from the blood that dramatically change the proportion and distribution of leukocytes in milk. However, the immune response to IMI is not uniform, since different leukocytes are altered according to the type of infecting pathogen and the duration of infection (Leitner *et al.*, 2012). For that reason it is very important to monitor the leukocyte distribution in milk that might be affected by the number of somatic cells and the presence of pathogen.

CONCLUSION

Proposed flow-cytometric approach can easily evaluate the composition of milk somatic cells. It seems that increased leukocytes, mainly polymorphonuclear cells, in the milk of ewes might be in relationship with the rise of somatic cell count and the presence of infectious bacteria species that could finally indicate the inflammation of mammary gland. However, further experiments with a large number of samples are required in order to confirm this hypothesis.

ACKNOWLEDGEMENTS

This work was supported by the grants of Slovak Research and Development Agency: APVV-15-0072 and Cultural and Educational Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences: KEGA 039SPU-4/2019.

REFERENCES

Albenzio, M., & Caroprese, M. (2011). Differential leucocyte count for ewe milk with low and high somatic cell count. *Journal of Dairy Research*, 78(1), 43–48. https://doi:10.1017/S0022029910000798

Albenzio, M., Santillo, A., Caroprese, M., Ruggieri, D., Ciliberti, M., & Sevi, A. (2012). Immune competence of the mammary gland as affected by somatic cell and pathogenic bacteria in ewes with subclinical mastitis. *Journal of Dairy Science*, 95(7), 3877–3887. https://doi:10.3168/jds.2012-5357.

Bagnicka, E., Winnicka, A., Jóźwik, A., Rzewuska, M., Strzałkowska, N., Kościuczuk, E., Prusak, B., Kaba, J., Horbańczuk, J., & Krzyżewski, J. (2011). Relationship between somatic cell count and bacterial pathogens in goat milk. *Small Ruminant Research*, 100(1), 72–77. https://doi.org/10.1016/j.smallrumres.2011.04.014.

Boulaaba, A., Grabowski, N., & Klein, G. (2011). Differential cell count of caprine milk by flow cytometry and microscopy. *Small Ruminant Research*, 97(1-3), 117–123. https://doi.org/10.1016/j.smallrumres.2011.02.002

Dosogne, H., Vangroenweghe, F., Mehrzad, J., Massart-Leen, A. M., & Burvenich, C. (2003). Differential leukocyte count method for bovine low somatic cell count milk. *Journal of Dairy Science*, 86(3), 828–834. https://doi.org/10.3168/jds.S0022-0302(03)73665-0

Gonzalo, C., Carriedo, J. A., Baro, J. A., & Primitivo, F. (1994). Factors influencing variation of test day milk-yield, somatic cell count, fat and protein in dairy sheep. *Journal of Dairy Science*, 77(6), 1537–1542. https://10.3168/jds.S0022-0302(94)77094-6

Holko, I., Tančin, V., Tvarožková, K., Supuka, P., Supuková, A., & Mačuhová, L. (2019). Occurence and antimicrobial resistance of common udder pathogens isolated from sheep milk in Slovakia. *Potravinarstvo Slovak Journal of Food Sciences*, 13(1), 258–261. https://doi.org/10.5219/1067

Kern, G., Traulsen, I., Kemper, N., & Krieter, J. (2013). Analysis of somatic cell counts and risk factors associated with occurrence of bacteria in ewes of different primary purposes. *Livestock Science*, 157(2-3), 597–604. https://doi.org/10.1016/j.livsci.2013.09.008

Leitner, G., Chaffer, M., Shamay, A., Shapiro, F., Merin, U., Ezra, E., Saran, A., & Silanikove, N. (2004). Changes in milk composition as affected by subclinical mastitis in sheep. *Journal of Dairy Science*, 87(1), 46–52. http://10.3168/jds.S0022-0302(04)73140-9

Leitner, G., Merin, U., Krifucks, O., Blum, S., Rivas, A. L., & Silanikove, N. (2012). Effects of intra-mammary bacterial infection with coagulase negative staphylococci and stage of lactation on shedding of epithelial cells and infiltration of leukocytes into milk: Comparison among cows, goats and sheep. *Veterinary Immunology and Immunopathology*, 147(3-4), 202–210. https://doi.org/10.1016/j.vetimm.2012.04.019

Li, N., Richoux, R., Perruchot, M. H., Boutinaud, M., Mayol, J. F., & Gagnaire, V. (2015). Flow Cytometry Approach to Quantify the Viability of Milk Somatic Cell Counts after Various Physico-Chemical Treatments. *PLoS ONE*, 10(12), e0146071. https://doi:10.1371/journal.pone.0146071

Maurer, J., & Schaeren, W. (2007). Udder health and somatic cell counts in ewes. *Agrarforschung Schweiz*, 14(4), 162-167. Retrieved from https://www.agrarforschungschweiz.ch/artikel/download.php?filename=2007_04_1266.pdf

Mehne, D., Drees, S., Schuberth, H. J., Sauter-Louis, C., Zerbe, H., & Petzl, W. (2010). Accurate and rapid flow cytometric leukocyte differentiation in low and high somatic cell count milk. *Milchwissenschaft*, 65(3), 235.

Paape, M. J. & Wiggans, G. R., Bannerman, D. D., Thomas, D. L., Sanders, A. H., Contreras, A., Moroni, P., & Miller, R. H. Monitoring goat and sheep milk somatic cell counts. *Small Ruminant Research*, 68(1-2), 114–125. https://doi.org/10.1016/j.smallrumres.2006.09.014

Persson, Y., Nyman, A. K., Söderquist, L., Tomic, N., & Waller, K. P. (2017). Intramammary infections and somatic cell counts in meat and pelt producing ewes with clinically healthy udders. *Small Ruminant Research*, 156, 66–72. https://doi.org/10.1016/j.smallrumres.2017.09.012

Rupp, R., Lagriffoul, G., Astruc, J. M., & Barillet, F. (2003). Genetic parameters for milk somatic cell scores and relationships with production traits in French Lacaune dairy sheep. Journal of Dairy Science, 86(4), 1476–1481. https://doi.org/10.3168/jds.S0022-0302(03)73732-1

Sarikaya, H., Prgomet, C., Pfaffl, M. W., & Bruckmaier, R. M. (2004). Differentiation of leukocytes in bovine milk. *Milchwissenschaft*, 59(11/12), 586–589. https://eurekamag.com/research/004/107/004107892.php

Silanikove, N., Shapiro, F., Leitner, G., & Merin, U. (2005). Subclinical mastitis affects the plasmin system, milk composition and curd yield in sheep and goats: comparative aspects. *Mastitis in Dairy Production*. Wageningen Academic Press Publishers, The Netherlands, 511–516.

Shosahni, E., Leitner, G., Hanochi, B., Saran, A., Shpigel, N. Y., & Berman, A. (2000). Mammary infection with *Staphylococcus aureus* in cows: progress from inoculation to chronic infection and its detection. *Journal of Dairy Research*, 67(2), 155–169. https://doi.org/10.1017/S002202990000412X

Souza, F. N., Blagitz, M. G., Penna, C. F. A. M., Della Libera, A. M. M. P., Heinemann, M. B., & Cerqueira, M. M. O. P. (2012). Somatic cell count in small ruminants: Friend or foe? *Small Ruminant Research*, 107(2-3), 65–75. https://doi.org/10.1016/j.smallrumres.2012.04.005

Świderek, W., Charon, K., Winnicka, A., Gruszczyńska, J., & Pierzchała, M. (2016). Physiological Threshold of Somatic Cell Count in Milk of Polish Heath Sheep and Polish Lowland Sheep. *Annals of Animal Science*, 16(1), 155–170. https://doi.org/10.1515/aoas-2015-0071

Tančin, V., Bauer, M., Holko, I., & Baranovič, Š. (2016). Etiology of mastitis in ewes and possible genetic and epigenetic factors involved. *Slovak Journal of Animal Science*, 49(2), 85–93. Retrieved from http://www.cvzv.sk/slju/16_2/tancin.pdf

Tančin, V., Holko, I., Vršková, M., Uhrinčať, M., & Mačuhová, L. (2017). Relationship between presence of mastitis pathogens and somatic cell count in milk of ewes. *XLVII. Lenfeldovy a Höklovy dny*. Brno: Veterinární a farmaceutická univerzita, 230–233. ISBN 978-80-7305-793-0.