Diagnosing mastitis in early lactation: use of Somaticell®, California mastitis test and somatic cell count

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ABSTRACT

The objective of the present study was to evaluate different methods for indirectly diagnosing mastitis during the postpartum period. These methods were: automatic and microscopic somatic cell counting (SCC), the California Mastitis Test (CMT) and Somaticell®. A total of 538 milk samples from 34 cows were used. These were collected at six times: day of parturition (M1) and 3 (M2), 7 (M3), 15 (M4), 21 (M5) and 30 (M6) days after parturition. Automatic and microscopic SCC, CMT and Somaticell® were all able to detect mastitis during the immediate postpartum period (up to 3 days postpartum). However, higher cut-off values should be applied to automatic and microscopic SCC. The negative score (score 0) of CMT was considered to be the best cut-off point at all times. Moreover, the values found using the Somaticell® test should not be used to presume the automatic SCC values, since there are discrepancies between the values of Somaticell® and automatic and microscopic SCC. It can be concluded that the different methods evaluated here to milk cellularity can be applied for diagnosing bovine mastitis, even during the immediate postpartum period, when there is greater cellularity, such as in the colostrum and transition milk.

INTRODUCTION

Mastitis is the most important disease to affect dairy herds because of its economic impact, which is caused by high prevalence, expenditure on medications, losses of production and milk quality, labour and, consequently, early discarding of animals. This disease is the main cause of use of antimicrobial agents on dairy farms. Their use may be related to development of antimicrobial-resistant strains that can threaten public health and this is corroborated by the possible presence of antimicrobial residues in milk and dairy products (Sargeant et al. 2001).

Mastitis can be classified in two ways. Regarding clinical signs, there are subclinical and clinical forms of mastitis. Clinical mastitis refers to cases in which the animals present evident physical manifestations in their mammary glands, which are easily noticed by the milk producer. However, subclinical mastitis is the predominant form of bovine mastitis and is characterised by absence of visible alterations in either the mammary gland or in the milk. Thus, diagnosing this form of bovine mastitis is crucial for minimising occurrences of the disease in dairy herds, through establishing adequate control measures that aim to reduce its impact throughout the milk production chain (Della Libera, Souza, et al. 2011; Mira et al. 2013).

Microbiological examination of milk is considered to be the standard method for diagnosing bovine mastitis, despite its limitations such as the time needed for culturing and the costs (Della Libera, Souza, et al. 2011; Souza et al. 2016). However, during the inflammatory process, there is significant migration of leukocytes to the area of inflammation. This makes it possible to use the cellularity of the milk to monitor the health of the animal’s mammary gland (Della Libera, Souza, et al. 2011). Among the indirect methods for diagnosing bovine mastitis, procedures such automatic and microscopic somatic cell counting (SCC), the California Mastitis Test (CMT) and Somaticell®, which evaluate milk cellularity, have been...
widely used (Sargeant et al. 2001; Rodrigues et al. 2009; Della Libera, Souza, et al. 2011; Langoni et al. 2012; Mira et al. 2013).

Somatic cell counting is a quantitative method for diagnosing mastitis, in which the different types of cells present in milk are enumerated (Souza et al. 2016). The California Mastitis Test (CMT) is an easy-to-perform, low-cost, fast test that can be performed on the farm, which despite its subjectivity is widely used during milking for diagnosing subclinical mastitis (Della Libera, Souza, et al. 2011; Mira et al. 2013). Somaticell® is a quantitative evaluation method, similar to the Wisconsin Mastitis Test, and was developed with the intention of reaching results similar to those of the SCC, but with the advantage that it can be used in the field (Rodrigues et al. 2009).

SCC cut-off values for diagnosing bovine mastitis have been established at between 100,000 and 272,000 cells mL⁻¹ (Schepers et al. 1997; Schukken et al. 2003; Bansal et al. 2005; Della Libera, Souza, et al. 2011; Souza et al. 2016). However, the cellularity of milk during the immediate postpartum period is high, even in uninfected mammary quarters (Gomes et al. 2011). This could have implications for using indirect methods that assess milk cellularity for diagnosing mastitis during the postpartum period, which is considered to be a period of high susceptibility to new intra-mammary infections (Pyörälä 2008). SCC is known to diminish more rapidly in healthy mammary quarters than in infected ones (Barkema et al. 1999). Similarly, Maunsell et al. (1999) and Sargeant et al. (2001) observed that the results from SCC and CMT in samples of milk/colostrum during the immediate postpartum period were significantly correlated with the health of the mammary gland. Therefore, studies on use of indirect methods for diagnosing bovine mastitis during the immediate postpartum period are scarce. Moreover, there are no reports in the literature regarding use of Somaticell® during the immediate postpartum period.

Thus, the objective of the present study was to compare the following different methods for diagnosing mastitis during the postpartum period: Somaticell®, microscopic SCC, automatic SCC and CMT.

**Materials and methods**

**Animals used and experiment design**

This research complied with the Ethical Principles in Animal Research and was approved by the Ethics Committee of the Faculty of Veterinary Medicine and Animal Science University of São Paulo (protocol number 9945030214).

A total of 538 quarter milk samples from 34 Holstein cows reared in a semi-intensive system were used in the present study. The milk samples were collected at six times: on the day of parturition (M1) and 7 (M2), 7 (M3), 15 (M4), 21 (M5) and 30 (M6) days after parturition. At all times, after discarding the three first gushes and performing antisepsis on the extremity of the teats, using cotton wool soaked in 70% alcohol, milk samples from each mammary quarter were collected for bacteriological examination, automatic SCC, microscopic SCC, CMT and Somaticell®.

**Bacteriological examination**

For the bacteriological examination, 0.01 mL of the milk samples was seeded onto Petri dishes containing 5% defibrinated sheep blood agar and was then incubated for 24–72 h at 37°C, followed by Gram’s test, observation of colony morphology and biochemical tests (Oliver et al. 2004). The milk sample was considered positive when ≥3 colonies grew, with the exception of samples that were positive for either *Staphylococcus aureus* or *Streptococcus agalactiae*, which were considered positive with growth of ≥1 colony (Piepers and De Vliegher 2009; Souza et al. 2016).

**Automatic somatic cell counting (automatic SCC)**

The samples used for automatic SCC were stored in flasks containing 2-bromo-2-nitropropane-1,3-diol and were transported to one of the accredited reference laboratories of the Ministry of Agriculture, Livestock-rearing and Supply (MAPA). Automatic SCC was performed there using the Somacount 300 automatic somatic cell counter (Bentley Instruments, Chaska, MN).

**Microscopic somatic cell counting (microscopic SCC)**

The milk samples used for microscopic SCC were assessed in accordance with the method proposed by Prescott and Breed (1910) and modified as described by Gomes et al. (2011) for M1 and M2 and by Della Libera et al. (2004) for M3, M4, M5 and M6. Slides were prepared in duplicates. After they had been dried and fixed in methanol, they were stained using Diff-Quik. The stained smears were examined using microscopy with magnification of 100×. Slide-reading yielded total and differential count results for mononuclear and polymorphonuclear leukocytes.
The CMT was performed as described by Schalm and Noorlander (1957). The results were interpreted as suggested by Kivaria et al. (2007) and were classified as follows: negative (score 0), trace (score 1), weakly positive (score 2), positive (score 3) and strongly positive (score 4).

Somaticell®

Somaticell® is an on-farm test that was performed in accordance with the manufacturer’s recommendations (Intervet Schering-Plough Animal Health, Whitehouse Station, NJ) and as previously detailed by Rodrigues et al. (2009) and Langoni et al. (2012). Briefly, this test consists of a single-use plastic graduated vial with a predetermined scale of SCC, a perforated cap, a straw for mixing and a reagent. For this test, 2 mL of milk was added into the plastic graduated vial followed by 2 mL of the reagent, and then mixed. Afterwards, the vial was then closed with the cap, and the vial was inverted for 30 s to allow non-coagulated solution to drain from the vial. Finally, the vial was returned to the upright position, and after 5 s, the value indicated on the vial SCC equivalent scale was recorded.

Statistical analysis

All the statistical analyses to determine predictive values (sensitivity, specificity and area under the curve of the receiver operating characteristics [ROC]) of the different inflammatory indicators were performed using the MedCalc statistical software (Ostend, Belgium). Calculations of the diagnostic tests characteristics were performed by using the milk bacteriological culture results as the gold standard (Dingwell et al. 2003). Sensitivity means to the proportion of individuals who have the target condition (reference standard positive) and give positive test results (i.e. ‘positivity in disease’). Specificity refers to the proportion of individuals without a disease that are negative in the test results (i.e. ‘negativity in health’). The area under the ROC curve enables the best cut-offs for clinical use to be given that maximised the sensitivity and specificity (Florkowski 2008). The SCC data were transformed to a logarithmic scale because they did not present normal distribution. The correlations among the different inflammatory indicators were determined by means of Pearson’s correlation (parametric data) and Spearman’s correlation (nonparametric data). Statistical significance was set at \( p < 0.05 \).

### Table 1. Predictive values of automatic and microscopic SCC for diagnosing bovine mastitis at different times during the postpartum period.

| Time (days postpartum) | Log automatic SCC | Log microscopic SCC |
|------------------------|-------------------|-------------------|
| M1 (n = 64)            | Cut-off value     | Cut-off value     |
|                        | 5.64              | 5.64              |
|                        | Sensitivity       | 77.78             |
|                        | Specificity       | 64.29             |
|                        | Area under ROC    | 0.74 (0.62–0.85)  |
| M2 (n = 89)            | Cut-off value     | 6.04              |
|                        | 4.36              | 5.00              |
|                        | Sensitivity       | 78.18             |
|                        | Specificity       | 64.33             |
|                        | Area under ROC    | 0.60 (0.49–0.70)  |
| M3 (n = 83)            | Cut-off value     | 6.54              |
|                        | 5.78              | 6.93              |
|                        | Sensitivity       | 79.18             |
|                        | Specificity       | 64.66             |
|                        | Area under ROC    | 0.59 (0.48–0.70)  |
| M4 (n = 100)           | Cut-off value     | 7.78              |
|                        | 5.78              | 21.43             |
|                        | Sensitivity       | 85.45             |
|                        | Specificity       | 80.00             |
|                        | Area under ROC    | 0.60 (0.50–0.70)  |
| M5 (n = 106)           | Cut-off value     | 6.93              |
|                        | 5.51              | 63.75             |
|                        | Sensitivity       | 87.33             |
|                        | Specificity       | 78.26             |
|                        | Area under ROC    | 0.59 (0.49–0.70)  |
| M6 (n = 94)            | Cut-off value     | 7.18              |
|                        | 4.15              | 69.00             |
|                        | Sensitivity       | 87.26             |
|                        | Specificity       | 78.26             |
|                        | Area under ROC    | 0.58 (0.47–0.69)  |
Results

The predictive values of the inflammatory indicators for diagnosing bovine mastitis at different postpartum times and the cut-off values that maximises the sensitivity and specificity of the test are presented in Tables 1 and 2. We observed that all indicators of inflammation evaluated here can be used to diagnose mastitis during the early lactation period considering their predictive values (Tables 1 and 2). Furthermore, although it appears puzzling, the predictive values of all diagnostic tests were greater during the immediate postpartum period than at subsequent times (Tables 1 and 2). However, higher cut-off values for SCCs should be used during the first 3 days after parturition (colostrum and transition milk), as shown by the cut-off values established from the area under the ROC curve for optimisation of sensitivity according to specificity (Table 1). The correlation between the different inflammatory indicators evaluated here at the postpartum period moments are positive and extremely significant, and summarised in Table 3. Finally, a large proportion of environmental pathogens were isolated from milk samples aseptically collected for bacteriological analysis during the early lactation, as showed in Table 4.

Discussion

The present study demonstrated that automatic and microscopic SCC, CMT and Somaticell® can all be used to diagnose bovine mastitis during the immediate postpartum period (even at parturition and 3 days after parturition) and determine from which milk quarter samples should be taken for bacteriological examination, since they presented good predictive values. The CMT score zero was considered to be the best cut-off value for diagnosing mastitis at all the times analysed and therefore for selecting animals for subsequent microbiological analysis on their milk, even during the period of greatest cellularity, such as in the colostrum and the transition milk, thus corroborating the findings of Sargeant et al. (2001) and Dingwell et al. (2003). Thus, milk quarter samples with CMT score higher than zero is suggestive to be infected.

Mononuclear leukocytes are known to predominate in colostrum and milk samples from healthy cows (Gomes et al. 2011; Blagitz et al. 2015). Moreover, higher CMT scores are not due simply to an increase in SCC, but also to alteration of the proportion of leucocytes in mammary gland secretions, which may be a result of increased neutrophils in infected mammary glands (Della Libera, Blagitz, et al. 2011). This could explain the results found in the present study, in

| M1 (n = 64) | M2 (n = 89) | M3 (n = 83) | M4 (n = 106) | M5 (n = 101) | M6 (n = 94) |
|------------|------------|------------|-------------|-------------|------------|
| CMT        |            |            |             |             |            |
| Cut-off value | 0          | 147        | 147         | 147         | 147        |
| Sensitivity | 0.88 (0.39)| 0.66 (0.32)| 0.66 (0.32)| 0.66 (0.32)| 0.66 (0.32)|
| Specificity | 0.57 (0.32)| 0.55 (0.31)| 0.55 (0.31)| 0.55 (0.31)| 0.55 (0.31)|
| Area under the ROC curve (95% Cl.) | 0.59 (0.49-0.69)| 0.55 (0.44-0.66)| 0.55 (0.44-0.66)| 0.55 (0.44-0.66)| 0.55 (0.44-0.66)|
| Somaticell® |            |            |             |             |            |
| Cut-off value | 0          | 0          | 0           | 0           | 0          |
| Sensitivity | 0.66 (0.32)| 0.66 (0.32)| 0.66 (0.32)| 0.66 (0.32)| 0.66 (0.32)|
| Specificity | 0.56 (0.31)| 0.55 (0.31)| 0.55 (0.31)| 0.55 (0.31)| 0.55 (0.31)|
| Area under the ROC curve (95% Cl.) | 0.59 (0.49-0.69)| 0.55 (0.44-0.66)| 0.55 (0.44-0.66)| 0.55 (0.44-0.66)| 0.55 (0.44-0.66)|

CMT: California Mastitis Test scores; M1: day of parturition; M2: 3 days after parturition; M3: 7 days after parturition; M4: 15 days after parturition; M5: 30 days after parturition; M6: 45 days after parturition; ROC: receiver operating characteristic; 95% CI: 95% confidence interval.
Table 3. Correlation between automatic and microscopic somatic cell counting, California Mastitis Test and Somaticell<sup>s</sup> at the different times during the postpartum period.

|          | Somaticell<sup>s</sup> | CMT | Automatic SCC | Microscopic SCC | Mononuclear leukocyte count |
|----------|------------------------|-----|---------------|-----------------|-----------------------------|
| M1       |                        |     |               |                 |                             |
| Somaticell<sup>s</sup> | –           | 0.57*| 0.69*          | 0.59*           | 0.62*                       |
| CMT      | –                      | 0.69*| 0.75*          | 0.61*           | 0.62*                       |
| Automatic SCC | 0.46*    | 0.69*| 0.59*          | 0.57*           | 0.57*                       |
| Microscopic SCC | 0.59*    |     | 0.59*          | 0.61*           | 0.61*                       |
| Mononuclear leukocyte count | 0.62* | 0.57*| 0.57*          | 0.61*           | 0.97*                       |
| Polymorphonuclear leukocyte count | 0.57* | 0.62*| 0.65*          | 0.94*           | 0.87*                       |
| M2       |                        |     |               |                 |                             |
| Somaticell<sup>s</sup> | –           | 0.67*| 0.75*          | 0.59*           | 0.64*                       |
| CMT      | –                      | 0.69*| 0.58*          | 0.72*           | 0.98*                       |
| Automatic SCC | 0.61*    | 0.69*| 0.66*          | 0.73*           | 0.91*                       |
| Microscopic SCC | 0.59*    |     | 0.66*          | 0.73*           | 0.85*                       |
| Mononuclear leukocyte count | 0.56* | 0.67*| 0.78*          | 0.78*           | 0.89*                       |
| Polymorphonuclear leukocyte count | 0.53* | 0.66*| 0.73*          | 0.91*           | 0.88*                       |
| M3       |                        |     |               |                 |                             |
| Somaticell<sup>s</sup> | –           | 0.67*| 0.75*          | 0.59*           | 0.64*                       |
| CMT      | –                      | 0.69*| 0.66*          | 0.73*           | 0.91*                       |
| Automatic SCC | 0.61*    | 0.69*| 0.66*          | 0.73*           | 0.91*                       |
| Microscopic SCC | 0.66*    |     | 0.66*          | 0.73*           | 0.91*                       |
| Mononuclear leukocyte count | 0.65* | 0.65*| 0.76*          | 0.78*           | 0.89*                       |
| Polymorphonuclear leukocyte count | 0.69* | 0.66*| 0.76*          | 0.93*           | 0.88*                       |
| M4       |                        |     |               |                 |                             |
| Somaticell<sup>s</sup> | –           | 0.62*| 0.66*          | 0.73*           | 0.63*                       |
| CMT      | –                      | 0.69*| 0.66*          | 0.66*           | 0.73*                       |
| Automatic SCC | 0.42*    | 0.66*| 0.73*          | 0.57*           | 0.64*                       |
| Microscopic SCC | 0.42*    |     | 0.58*          | 0.66*           | 0.64*                       |
| Mononuclear leukocyte count | 0.40* | 0.58*| 0.66*          | 0.73*           | 0.57*                       |
| Polymorphonuclear leukocyte count | 0.40* | 0.58*| 0.66*          | 0.73*           | 0.57*                       |
| M5       |                        |     |               |                 |                             |
| Somaticell<sup>s</sup> | –           | 0.62*| 0.66*          | 0.73*           | 0.63*                       |
| CMT      | –                      | 0.66*| 0.73*          | 0.66*           | 0.73*                       |
| Automatic SCC | 0.59*    | 0.66*| 0.73*          | 0.66*           | 0.73*                       |
| Microscopic SCC | 0.59*    |     | 0.66*          | 0.66*           | 0.73*                       |
| Mononuclear leukocyte count | 0.56* | 0.66*| 0.66*          | 0.73*           | 0.74*                       |
| Polymorphonuclear leukocyte count | 0.56* | 0.66*| 0.66*          | 0.73*           | 0.74*                       |
| M6       |                        |     |               |                 |                             |
| Somaticell<sup>s</sup> | –           | 0.70*| 0.66*          | 0.66*           | 0.66*                       |
| CMT      | –                      | 0.66*| 0.66*          | 0.66*           | 0.66*                       |
| Automatic SCC | 0.54*    | 0.66*| 0.71*          | 0.66*           | 0.66*                       |
| Microscopic SCC | 0.54*    |     | 0.68*          | 0.66*           | 0.66*                       |
| Mononuclear leukocyte count | 0.60* | 0.66*| 0.71*          | 0.66*           | 0.66*                       |
| Polymorphonuclear leukocyte count | 0.59* | 0.66*| 0.69*          | 0.69*           | 0.78*                       |

SCC: somatic cell count; CMT: California Mastitis test; M1: day of parturition; M2: 3 days after parturition; M3: 7 days after parturition; M4: 15 days after parturition; M5: 21 days after parturition; M6: 30 days after parturition; ROC: receiver operating characteristics; *p ≤ 0.0001.

Table 4. Results from bacteriological examination of the milk samples from the mammary quadrants at different times during the postpartum period.

| Pathogens isolated | M1 (n = 64) | M2 (n = 89) | M3 (n = 83) | M4 (n = 106) | M5 (n = 101) | M6 (n = 94) |
|--------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Negative           | 55 (85.94%) | 75 (84.27%) | 73 (87.95%) | 92 (86.80%) | 80 (79.21%) | 80 (85.11%) |
| Staphylococcus epidermidis | 3 (4.06%) | 3 (3.73%) | 4 (4.06%) | 3 (2.08%) | 4 (3.96%) | 2 (2.13%) |
| Staphylococcus haemolyticus | 1 (1.56%) | 2 (2.25%) | 2 (2.42%) | 2 (1.90%) | 3 (2.96%) | 1 (1.06%) |
| Staphylococcus hyicus | 1 (1.56%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) |
| Staphylococcus chromogenes | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) |
| Staphylococcus aureus | 0 (0.00%) | 2 (2.86%) | 1 (1.20%) | 1 (0.94%) | 2 (1.98%) | 0 (0.00%) |
| Streptococcus uberis | 2 (3.13%) | 5 (5.62%) | 1 (1.20%) | 1 (0.94%) | 1 (0.99%) | 1 (1.06%) |
| Streptococcus agalactiae | 1 (1.56%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 3 (2.97%) | 2 (2.13%) |
| Corynebacterium bovis | 0 (0.00%) | 1 (1.12%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) |
| Micrococcus sp. | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 3 (2.83%) | 0 (0.00%) |
| Pseudomonas sp. | 1 (1.56%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 1 (0.94%) | 2 (1.98%) |
| Klebsiella sp. | 0 (0.00%) | 1 (1.12%) | 1 (1.20%) | 0 (0.00 %) | 1 (0.99%) | 2 (2.13%) |
| Escherichia coli | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 2 (1.89%) | 0 (0.00%) | 0 (0.00%) |
| Contaminated | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 2 (1.98%) | 0 (0.00%) |

M1: day of parturition; M2: 3 days after parturition; M3: 7 days after parturition; M4: 15 days after parturition; M5: 21 days after parturition; M6: 30 days after parturition.
which the CMT score of zero was found to be the cut-off value at all the times assessed.

Sensitivity and specificity were considered to be equally important in the present study for determining the best cut-off value using the area under the ROC curve, as proposed by Detilleux et al. (1999) and Sargeant et al. (2001). The predictive values of CMT and Somaticell® were slightly lower than those of the automatic and microscopic SCC, especially during the immediate postpartum period. However, CMT and Somaticell® have the advantage of being easy to use and quick to apply in the field.

Similarly to our findings, Sargeant et al. (2001) demonstrated that different methods for evaluating milk cellularity (in this case CMT and SCC) can be used to detect mastitis and to screen for mammary quarters that should be sampled for milk/colostrum, in order to make bacteriological diagnoses of intra-mammary infections during this period of high susceptibility. This result was supported in the present study by the positive and extremely significant correlation that was found between the different methods assessing milk/colostrum cellularity.

The correlation coefficients between Somaticell® and automatic SCC (0.46–0.69) were higher than those described by Langoni et al. (2012), but lower than those of Rodrigues et al. (2009). However, most of the milk samples in the study by Rodrigues et al. (2009) presented low SCC, unlike the present study and the study by Langoni et al. (2012), which suggests that Somaticell® presents better performance in milk samples with low SCC. Moreover, according to the findings of the present study, the cut-off values found using the Somaticell® test results based on area under the ROC curve should not be used to presume the values from logarithm automatic SCC. Thus, discrepancies were observed between the cut-off values of Somaticell® and those of automatic and microscopic SCC, especially during the immediate postpartum period.

Conversely, the cut-off values of SCC fell rapidly 3 days after parturition and remained within the widely used and recommended values (between 100,000 and 200,000 cells mL⁻¹) for diagnosing bovine mastitis (Della Libera, Souza, et al. 2011, Schepers et al. 1997; Schukken et al. 2003; Bansal et al. 2005). In addition, considerable prevalence of environmental pathogens such as Gram-negative bacteria (e.g. Pseudomonas sp., Klebsiella sp. and Escherichia coli), environmental streptococci (e.g. S. uberis) and coagulase-negative staphylococci (e.g. S. haemolyticus) were observed among the pathogens isolated from the milk samples in the postpartum period. This finding corroborates the hypothesis that the greatest problem in the aetiology of mastitis during the postpartum period relates to environmental pathogens (Pyörälä 2008). On the other hand, the prevalences of contagious pathogens (e.g. S. aureus, S. agalactiae, S. chromogenes, S. epidermidis and C. bovis) were relatively low, in comparison with other studies in Brazil (Souza et al. 2009; Oliveira et al. 2011; Souza et al. 2016).

A positive correlation was observed between the methods for evaluating milk cellularity and the mononuclear and polymorphonuclear leukocyte counts. This suggests that the increase in milk cellularity during the inflammatory process is not restricted only to infiltration of polymorphonuclear leukocytes, such as the increased levels of CD8+ T lymphocytes that have been described for mammary quarters infected by Staphylococcus aureus and Streptococcus dysgalactiae (Park et al. 1993; Blagitz et al. 2015).

These parameters can be influenced by factors such as age, lactation stage, production, seasons, dairy fraction collected, variations among animals and pathogenicity of the agent, which can be reflected in the evaluation of the tests analysed. Moreover, a large proportion of the prediction of diagnostic tests is based on having a bacteriological examination as the gold standard. However, mastitis does not always require the presence and isolation of bacteria is not always successful (Della Libera, Souza, et al. 2011; Souza et al. 2016). Thus, some pathogens that are considered to be secondary, such as Corynebacterium bovis and some coagulase-negative Staphylococcus species, usually do not lead to an increase in milk cellularity that goes above the cut-off values established for the SCC (Djabri et al. 2002; Souza et al. 2009, 2016). On the other hand, the absence of bacterial isolation in milk samples with high SCC may indicate that low numbers of pathogens are being eliminated in the milk. This would reduce the likelihood of isolation, which may be due to effective control of the infection by the leukocytes present in the milk (Souza et al. 2016).

Conclusions

The different methods used here to assess milk cellularity has a potential to be used to diagnose bovine mastitis and to select optimal sampling strategy to determine the mammary quarters from which milk samples should be taken for microbiological examination, even for fresh cows, which presents greater cellularity. However, higher values for microscopic and automatic SCC should be used, while a negative score (score 0) could be used for the CMT at all times. Somaticell® may also be used, but its quantitative cut-off values should not be used to presume the values of logarithm SCC, especially during the immediate postpartum period.
Disclosure statement

The authors report no conflicts of interest. The authors are responsible for the content and writing of this paper.

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