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Physical, laboratory, and microbiological parameters of mammary gland secretions in postpartum does

Parâmetros físicos, laboratoriais e microbiológicos de secreções lácteas de cabras no período pós-parto

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Abstract

Mastitis is a multifactorial disease whose prevalence is affected by the type of pathogenic agent involved, the constitution of the animal, and environmental conditions. This study evaluated the physical and laboratory characteristics of colostrum from dairy goats in the postpartum period, and the physical characteristics of the mammary glands in the same period. Of the 71 mammary glands evaluated, 12 were positive for bacterial isolates and the most frequent pathogenic agents were coagulase-negative staphylococci (CNS) (n = 11). Median somatic cell counts (SCC) in animals positive for bacterial isolates were greater than in animals without bacterial isolates at parturition (696.0 vs. 256.0 x 10³ mL⁻¹) and 48 h postpartum (1,350 vs. 437.0 x 10³ mL⁻¹). In addition, 34 samples were positive for the California Mastitis Test (CMT; score >1+), indicating a positive relationship between this test and bacterial isolation. Coagulase-negative staphylococci (CNS) were the most prevalent pathogenic agents, resulting in increase in SCC. Postpartum changes in mammary secretions were not good predictors of bacterial mastitis. Physical examination of mammary glands did not reveal significant changes for the diagnosis of mastitis in the postpartum period.

Key words: Goats. Colostrum. Udder examination. Mastitis. Coagulase-negative staphylococci (CNS).

Resumo

A mastite é uma doença multifatorial que sofre influência do tipo de patógeno envolvido, constituição dos animais e condições ambientais. Foi realizado estudo com objetivo de avaliar as características físicas e laboratoriais do colostro de cabras no período pós-parto, assim como características físicas das glândulas mamárias no mesmo período. As glândulas mamárias foram submetidas a avaliações físicas que não resultaram em alterações significativas ao longo dos momentos pesquisados. Das 71 metades mamárias analisadas, 12 apresentaram isolamento microbiológico e o agente mais frequente foi *Staphylococcus* coagulase negativo (n=11). A mediana da contagem de células somáticas (CCS) dos animais positivos à lactocultura foi maior do que daqueles que não tiveram isolamento microbiano nas colheitas realizadas no momento do parto (696 x 10³ mL⁻¹) e após 48h (1.350 x 10³ mL⁻¹). O *California Mastitis Test* (CMT) mostrou-se positivo (>1+) em 34 amostras analisadas logo após o parto, revelando uma associação entre o referido teste e o isolamento microbiano. O grupo de agentes mais prevalente no

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período pós-parto foi *Staphylococcus* coagulase negativo, resultando em aumento na CCS. Alterações nas secreções lácteas vistas nesse período não podem ser consideradas como preditoras de mastites bacterianas. O exame físico das glândulas mamárias não revelou alterações relevantes para o diagnóstico de mastite no referido período.

Palavras-chave: Caprinos. Colostro. Exame do úbere. Mastite. Staphylococcus coagulase negativo.

Introduction

Mastitis is a multifactorial illness characterized by inflammation of the mammary gland. It that can be diagnosed based on changes in physical characteristics of the udder and/or its secretion (SMITH; SHERMAN, 1994). In small ruminants, intramammary infections are mostly caused by bacteria (BERGONIER et al., 2003). However, lentiviruses may also be involved in the occurrence of the disease and in the increase of somatic cell counts in milk (TURIN et al., 2005).

Mastitis can occur in a clinical and subclinical (asymptomatic) form. The clinical form is characterized by pathological signs in the udder and quantitative (decrease or absence) and qualitative (macroscopic aspect and composition) changes in milk secretion. In subclinical mastitis, intramammary infection evolves without clinical signs (MAROGNA et al., 2012), but milk yield is reduced.

The incidence of mastitis in sheep and goats is generally reported to be less than 5%. However, the prevalence of asymptomatic mastitis in small ruminants has been estimated to range between 5 and 30% (BERGONIER et al., 2003; CONTRERAS et al., 2003). In Brazil, the incidence of asymptomatic mastitis in goat herds ranges from 22 to 75% of animals (LIMA JÚNIOR et al., 1995).

Several microorganisms are frequently isolated in cases of asymptomatic mastitis in small ruminants, including *Staphylococcus epidermidis*, *S. chromogenes*, *S. simulans*, *S. caprae*, and *S. agalactiae* (CONTRERAS et al., 2003). Nevertheless, coagulase-negative staphylococci (CNS) are the main causative agents.

In dairy goats, some factors are associated with the occurrence of clinical or subclinical mastitis, including animal age, number of parturitions, litter size in each parturition, and lactation stage (ISLAM et al., 2011). In goats, there is a higher incidence of the disease at the beginning of lactation (SÁNCHEZ et al., 1999), whereas intramammary infections in the dry period or immediately postpartum are unusual and generally associated with fungal infections resulting from contamination or environments with poor hygiene practices (PÉREZ et al., 1998).

Diagnosis can be established by direct and indirect methods. The somatic cell count (SCC) and California Mastitis Test (CMT) are indirect methods, whereas isolation and polymerase chain reaction (PCR) amplification are direct methods (PATERNA et al., 2014). In goats, SCC increases during mammary infection and this increase is more pronounced in infections caused by major pathogenic bacteria such as *Staphylococcus aureus* and gram-negative bacilli, including *Escherichia coli*, *Pseudomonas* spp., *Mycoplasma* spp., *Proteus* spp., *Streptococcus* spp. (PAAPE et al., 2001), and *Trueperella pyogenes* (CAFFARO et al., 2014), than in infections caused by minor pathogens such as CNS.

Noninfectious factors such as calving, stage of lactation, year-seasons, and milk yield also affect SCC and may be responsible for 90% of the variation in SCC (WILSON et al., 1995). Moreover, some cytological characteristics of the goat species are important in determining SCC. Milk secretion in the goat is apocrine, resulting in the release of cytoplasmic nucleated particles into milk, which interfere with the SCC (PAAPE; CAPUCO, 1997).

This study aimed to determine the occurrence of mastitis by physical, laboratory, and microbiological assessments and to evaluate the diagnostic methods used immediately after delivery in dairy goats farmed in an intensive production system.

Materials and Methods

Animals

Thirty six Saanen (n = 23) and Brown-Alpine (n = 13) postpartum does randomly chosen from a single dairy property located in São José do Rio Preto, northwest of the state of São Paulo, Brazil, were evaluated. The farm had a herd of approximately 400 head. Samples were collected in March, July, and November, which corresponded to the parities in the property.

Handling

The animals were kept in confinement, housed in collective cages with folding slatted floors, and given access to a solarium. The facilities were not disinfected frequently.

Animals were fed corn silage, tifton grass hay, and commercial feed according to milk yield and lactation stage of each animal. Water and mineral salt were given *ad libitum*. Does produced milk for approximately 305 days, and then entered the dry period, which lasted approximately 60 days. Drying-off was done abruptly using intramammary antibiotics.

Does were dewormed immediately after parturition and all animals in the farm were vaccinated for the main clostridial diseases every four months, whereas caseous lymphadenitis was controlled by draining of abscesses only. However, carrier animals were not isolated. For control of caprine arthritis encephalitis (CAE), kids were isolated after birth and raised on pasteurized colostrum.

Macroscopic evaluation of colostrum samples

Kids born of experimental does were isolated immediately after birth for collection of colostrum samples from the mammary gland of recently calved does. The first three squirts were stored in a container with a dark background for evaluation of color, viscosity, opacity, and presence or absence of lumps immediately after parturition (M0) and 24 h (M24), and 48 h (M48) postpartum.

Harvest and procedures for microbiological analysis of colostrum

For microbiological analysis, an antiseptic colostrum sample from each half gland was taken immediately after delivery. Before harvest, the glands, and particularly the teat orifices, were cleaned according to the standards recommended by the International Dairy Federation (1981). After antisepsis, samples containing approximately 3 ml of colostrum from each gland were collected in sterile 15-ml Falcon tubes and sent for microbiological analysis at the School of Veterinary Medicine, São Paulo State University (UNESP), Araçatuba, SP, Brazil.

The samples were gently homogenized, plated on agar base media supplemented with defibrinated horse blood and MacConkey agar, and incubated at 37 °C under aerobic and microaerophilic conditions in a bacteriological incubator for at least 72 h. Readings were performed daily, after 24, 48, and 72 h of incubation, when the morphological macroscopic characteristics of the colonies, including size, color, pigmentation, and presence or absence of hemolysis halo were observed. Positive cultures were those in which at least three identical colonies grew in the same sample in culture medium. Next, gram stained smears were prepared from samples of the colonies for examination of micromorphological characteristics of bacteria. The microorganisms were identified according to Quinn et al. (2011). Experimental animals were divided into two groups: group 1 (No bacterial isolates, NBI), consisting of goats negative for bacterial isolates in glands, and group 2 (Bacterial isolates, BI), consisting of animals positive for bacterial isolates in at least one gland.

California Mastitis Test (CMT) and somatic cell count (SCC)

CMT analysis was performed using a neutral anionic detergent (Reagente CMT, FATEC Indústria de Nutrição e Saúde Animal Ltda., Arujá, SP, Brazil) and results were interpreted according to Schalm et al. (1971). Samples were considered positive when they reacted to more than one cross score. A sample with milk mixture from the two glands totaling 10 ml was collected for the somatic cell count and analyzed using a portable DeLaval cell counter DCC (DeLaval®, Hamra Gård, Sweden).

CMT and SCC were performed immediately after delivery (M0) and at 24 (M24) and 48 hours (M48) after delivery.

Physical examination of the mammary gland

Mammary glands were examined at the end of milking and evaluated according to their size, consistency, sensitivity, and temperature. Consistency was classified as pasty, firm, or hard; size was considered larger or smaller than expected. Temperature was classified as normal, hot, or cold, and sensitivity was characterized as increased or decreased relative to a painful stimulus.

These evaluations were performed immediately after parturition (M0) and at 24 (M24) and 48 hours (M48) postpartum.

Ethical aspects and biosafety

All experimental procedures were approved by the Ethics Committee on Animal Use (CEUAC-FOA) under protocol number 2013-01450.

Statistical analysis

Association tests were performed using the chi-square test. Differences between groups were analyzed using the Friedman test to determine

the effect of time and the Mann-Whitney test to determine the differences between groups. Data were checked for normality using the Kolmogorov-Smirnov test. All analyses were performed using Prism v.6.0 software (GraphPad Software Inc., San Diego, CA, USA). Data were considered significant at p < 0.05.

Results and Discussion

Thirty-six animals were used in the study, but only 71 mammary glands were examined because one gland did not have the teat orifice (atresia) and was not included in the analyses.

The results of the physical examination of udders with and without microbial isolation are shown in Table 1. One gland presented cutaneous nodules of floating consistency in the region corresponding to the teat canal. No significant changes were observed in glands without microbial isolation over the study period. However, 19 (32.2%) glands in the NBI group showed increased temperature and 22 (37.3%) glands were larger than expected at M0. Consistency was normal and characterized as pasty in 56 (94.9%) mammary glands in the NBI group. None of the glands that were positive for bacterial culture showed firm consistency at M0 and M24, whereas five (41.7%) of the 12 glands in the BI group were larger than expected at M0.

Few studies have investigated the physical characteristics of the mammary gland in the diagnosis of mastitis, especially in does. However, the presence of teat lesions and the shape of the teat ends may facilitate the occurrence of clinical mastitis (AMEH; TARI, 1999). Marogna et al. (2012) reported that 30.6% of milk samples taken from goats with clinical signs of udder infection were positive for bacteria. In the same study, goats with clinical signs of infection were 3.71 times more likely to be positive in milk sample cultures than goats without clinical signs of mastitis.

Table 1. Physical examination of mammary glands from dairy goats negative (NBI) and positive (BI) for bacterial isolates immediately after parturition and 24 and 48 h postpartum. São José do Rio Preto, SP, 2013.

Parameter	NBI			BI			
	0 h	24 h	48 h	0 h	24 h	48 h	
Consistency							
pasty	56	50	52	12	12	11	
firm	3	8	6	0	0	1	
hard	0	1	1	0	0	0	
Edema							
present	5	7	6	1	1	1	
absent	54	52	53	11	11	11	
Sensitivity							
regular	51	55	56	11	12	11	
increased	8	4	3	1	0	1	
absent	0	0	0	0	0	0	
Temperature							
regular	39	43	35	10	9	8	
increased	19	16	24	2	3	4	
decreased	1	0	0	0	0	0	
Size							
regular	37	46	54	7	9	9	
increased	22	13	5	5	3	3	
decreased	0	0	0	0	0	0	

The bagging up of mammary glands and teats as calving approaches is a physical sign of calving that is common to all domestic animals (JAINUDEEN; HAFEZ, 2004). Additionally, udder edema can also occur occasionally in dairy goat breeds before or after calving (ANDERSON et al., 2004).

In this study, mammary glands were examined immediately after parturition and at the following 48 hours. Therefore, some of the changes observed may have occurred because of calving and animals positive for bacteria had few changes in the udder on physical examination.

Of the 59 colostrum samples from glands of animals in the NBI group evaluated immediately after parturition (Table 2), 15 (21.1%) had lumps, whereas at 24 and 48 h lumps were detected in 12 samples (20.33%). Most samples in the NBI group were whitish (18/59, 30.5%) and yellowish (34/59, 57.6%) at M0 and two samples (3.4%) were colorless. Additionally, lumps were observed in

just three (25%) of the 12 colostrum samples from mammary glands of animals in the BI group (Table 2), indicating the occurrence of clinical mastitis. All samples positive for bacteria were opaque, and most of which (10/12, 83.3%) had a yellowish color.

Twelve (16.9%) of the 71 colostrum samples analyzed were positive for bacteria. Of these, nine (12.7%) were collected from animals with asymptomatic mastitis, whereas only three (4.2%) samples were from symptomatic animals. CNS was the most frequently isolated bacterial pathogen both in asymptomatic and symptomatic cases of mastitis. In addition, there was no association between the occurrence of mastitis and the presence of lumps in the milk secretion (p = 0.9755). This finding may have resulted from aseptic inflammation of the mammary gland and/or the presence of other viral and bacterial agents that were not investigated. Similarly, there was no significant difference concerning isolation between primiparous (n = 13)and pluriparous (n = 23) does (p = 0.6287).

Table 2. Macroscopic characteristics of milk from dairy goats negative (NBI) and positive (BI) for bacterial isolates immediately after parturition and 24 and 48 h postpartum. São José do Rio Preto, SP, 2013.

Parameter	NBI			BI		
	0 h	24 h	48 h	0 h	24 h	48 h
Lumps						
present	15	9	9	3	3	1
absent	44	50	50	9	9	11
Color						
whitish	18	52	58	2	8	12
brownish	5	0	0	0	0	0
yellowish	34	6	0	10	4	0
colorless	2	1	1	0	0	0
Opacity						
present	57	58	58	12	12	12
absent	2	1	1	0	0	0
Viscosity						
slightly viscous	37	58	58	11	11	12
viscous	20	0	0	1	1	0
aqueous	2	1	1	0	0	0

Some management practices in the farm may have influenced the occurrence of mastitis in this study, including cessation of milking at dry-off. Goats at the end of the lactation period were not dried-off gradually. In high-producing cows, gradual drying-off considerably reduced the amount and duration of milk leakage and the risk of mastitis at dry-off (ZOBEL et al., 2013). In the current study, the high number of animals per pen and poor hygiene practices in the facilities may also have contributed to the occurrence of mammary infections.

Several studies have been conducted in Brazil to determine the prevalence of mastitis in goats. Neves et al. (2010) reported a prevalence of 11.5% of culture-positive subclinical mastitis in the semi-arid of the state of Paraíba. Schmidt et al. (2009) reported 15.6% of culture-positive samples and found a higher incidence of the disease in does with lactation periods between eight and 12 months, but no cases of asymptomatic mastitis in animals lactating for less than seven days. Muricy (2003) reported a prevalence of 57.4% (74/129) of positive samples in the state of Minas Gerais, whereas White and Hinckley (1999) reported a prevalence of 36.4%

for clinical and subclinical mastitis and found 43.9% of samples with no microbial isolation, but increased SCC.

In this study, 16.9% of colostrum samples were positive for bacteria. However, it should be noted that these mammary infections probably occurred during the dry period. In small ruminants, the occurrence of mammary infections during the dry period has been poorly studied and, in general, is associated with contamination or poor hygiene practices (PÉREZ et al., 1998). Despite the low prevalence, mammary gland infections have been reported in dry ewes, and the main causative agents in these cases were CNS and Arcanobacterium pyogenes (SARATSISA et al., 1998), the latter recently renamed Trueperella pyogenes (CAFFARO et al., 2014). Moreover, evidence suggests that up to 61% of mammary infections in dairy goats persist during the dry period (LERONDELLE; POUTREL, 1984).

CNS are the most prevalent pathogens in subclinical mastitis in dairy animals (CONTRERAS et al., 2007). In Brazil, CNS have also been shown to be important pathogenic agents (CASTRO et

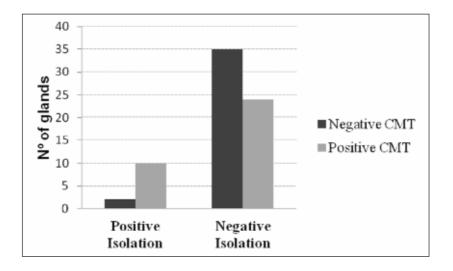
al., 1992; NEVES et al., 2010; PEIXOTO et al., 2010). Even though they have been described as less pathogenic than *S. aureus*, CNS are also able to persist in the mammary gland, causing a moderate increase in SCC. Moreover, their antimicrobial resistance is estimated to be much greater compared to that of *S. aureus* (TAPONEN; PYORALA, 2009). The current study also showed a higher prevalence of CNS, and only one case was caused by a different agent (*Bacillus* sp.).

Thirty-four (47.88%) samples were CMT positive, of which 10 (14.08%) had bacterial isolates (Figure 1), whereas only two (2.81%) samples were negative for CMT and positive in

milk sample cultures. Finally, 35 (49.25%) samples were negative for both CMT and bacterial culture. Importantly, there was a positive association between CMT positivity and microbiological isolation immediately after calving (p = 0.007).

There was no significant difference in the percentage of secretions with bacterial isolates between CMT positive and negative samples at M24 and M48. However, 30 (42.3%) secretions were negative both in milk sample cultures and the CMT at M24, and 29 samples (40.8%) were CMT positive but had no bacterial isolates. The results obtained at 48 h were similar to those obtained at 24 h.

Figure 1. California Mastitis Test (CMT) reactivity (positive samples > 1+) in colostrum samples from goats with and without bacterial isolates immediately after parturition. São José do Rio Preto, SP, 2013.



Our study revealed a relationship between CMT positivity (1 and 2 +) and the isolation of infectious agents. CMT had a sensitivity and specificity of 83.3% and 59.3%, respectively, and similar results were reported by McDougall et al. (2010). Even though bacteriological examination is considered the "gold standard" for diagnosis of mammary infections, the CMT has a role as a screening test given its low cost and reasonable sensitivity. Nevertheless, a confirmatory diagnosis test is needed. Conversely, Neves et al. (2010) did

not observe a similar association and concluded that CMT results are affected by lactation stage and therefore is not reliable for the diagnosis of subclinical mastitis in goats at different stages of lactation.

Median SCC (x 10^3 cells mL⁻¹) values are shown in Table 3. SCC in the NBI (p = 0.5818) and BI (p = 0.6293) groups were not significantly different over time. In addition, SCC was significantly higher in the BI group than in the NBI group at M0 (p = 0.0355) and M48 (p = 0.0023).

Table 3. Median and percentiles of somatic cell count (SCC x 10^3 cells mL⁻¹) in colostrum samples from dairy goats positive (NBI, n = 24) and negative (BI, n = 12) for bacterial isolates immediately after parturition and 24 and 48 h postpartum. São José do Rio Preto, SP, 2013.

Time -		NBI			BI			
	Median	P ₂₅	P ₇₅	Median	P ₂₅	P ₇₅		
0 h	256.0Aa	79.5	930.0	696.0Ab	486.0	1845.0		
24 h	453.0Aa	267.5	1274.5	1051.0Aa	576.0	2747.0		
48 h	437.0Aa	246.25	850.5	1350.0Ab	702.0	2300.0		

Medians followed by different lowercase letters in the same row and uppercase letters in a column are significantly different (p < 0.05).

For economic reasons and because milk from both mammary glands, even in cases of asymptomatic mastitis, is mixed for processing, SCC of composite milk samples is routine practice in milk-producing farms. Thus, we conducted SCC of composite samples to replicate the usual handling practices of dairy farms. Of the 71 glands evaluated in this study, only three showed macroscopic changes in milk and were positive on bacteriological examination, indicating the difficulty in diagnosing mastitis at this stage of lactation. This finding emphasizes the importance of early diagnostic measures, because undetected changes in milk secretion can affect the cell count of all the milk produced.

The mixture of high SCC milk from infected mammary glands with low SCC milk from healthy glands is insufficient to reduce total SCC in the bulk milk tank. Even though goats and sheep only have two mammary glands, the SCC from the uninfected gland does not minimize the effect of the SCC from the infected gland (LEITNER et al., 2008).

SCC values were also compared between primiparous and pluriparous does. Negative pluriparous does on milk sample culture (n = 14) showed higher SCC than negative primiparous does (n = 10) at parturition (p = 0.007). This result is in agreement with the findings of Wilson et al. (1995), who also demonstrated an association between the number of lactations and increased SCC.

Somatic cell counts in milk from goats are naturally higher than SCC in milk from cows and increase with lactation stage because of the type (apocrine) of milk secretion in goats (PAAPE; CAPUCO, 1997). Polymorphonucleated cells are the most predominant type of white blood cells in goat milk, and neutrophils alone make up to 70% of the SCC in goat milk, whereas they only make up to 3 to 26% of the total cell count in bovine milk. Because neutrophils are the first line of immune defense, this may explain the greater resistance of goats to mammary infections relative to cows (TIAN et al., 2005).

Other non-infectious factors such as calving (DULIN et al., 1983), stage of lactation (CUCCURU et al., 1997), type of food (PAAPE et al., 2007), year-seasons (MCDOUGALL et al., 2001), milk yield (MCDOUGALL et al., 2001), milking frequency (NUDDA et al., 2002), and manual or mechanical milking (SINAPIS, 2007) can also affect SCC.

Median SCC of animals in the BI and NBI groups were not significantly different over the study periods. However, animals that were positive in the microbiological test had a higher SCC at M0 than at M24 and M48 (p = 0.0355). Moreno-Indias et al. (2012) found average SCC values of 5,819 x 10³ cells mL⁻¹ in colostrum samples not subjected to bacteriological culture immediately after parturition. Sánchez-Macías et al. (2014) evaluated the physical, chemical, and immune parameters of goat colostrum and reported average SCC values of 8,44 x 10³ cells mL⁻¹ in colostrum samples immediately after parturition and 6,539 x 10³ and 4,624 x 10³ cells mL⁻¹, 24 and 48 h later,

respectively. McDougall et al. (2010) found higher SCC values in infected mammary glands than in uninfected glands. The SCC values reported by these authors were approximately 6,000 x 10³ cells ml⁻¹ for positive milk sample cultures between the time of parturition and one day postpartum. However, the SCC values from milk samples negative for bacterial isolates were lower (< 1,000 x 10³ cells mL⁻¹) than the SCC values reported elsewhere.

Fluoroscopy methods have been used for determination of SCC in goats because they are specific for the detection of DNA. In this study, a different methodology was used for counting somatic cells (DeLaval cell counter DCC, DeLaval®). However, Berry and Broughan (2007) found a correlation between cell counts using a DCC counter and direct microscopy, which is the reference method for cell counts in dairy goats. Similarly, Moreno-Indias et al. (2012) also used the DCC counter in colostrum samples for the determination of somatic cells in goats.

The difference between SCC values in this study and the literature may be explained by factors such as the statistical model and methodology used for SCC determination, animal breed, age, breeding system, environment, year-seasons, type of bacterial isolate, and handling procedures adopted in the drying-off process.

SCC can also be significantly affected by non-infectious factors. Souza et al. (2009) reported that the composition and SCC of milk from dairy goats were significantly affected by herd, season, and milking system. In that study, 13% of the variation in SCC was explained by season, with lower SCC values observed at early lactation (winter) and higher SCC values at the end of lactation (summer). McDougall et al. (2010) observed that infected glands treated with antibiotics had lower SCC than untreated glands in postpartum dairy goats. This fact may partly explain the difference in cell counts between the current study and the literature, because all animals in this study were treated with

intramammary antibiotics at the time of drying-off. It should be noted that even though this drying-off technique has proven effective, its use is not widespread in dairy goats.

In conclusion, coagulase-negative staphylococci (CNS) were the most prevalent pathogens isolated from the milk of postpartum dairy goats in this study, resulting in increased SCC. Moreover, postpartum changes in mammary secretions are not good predictors of bacterial mastitis. Finally, physical examination of mammary glands did not reveal significant changes for the diagnosis of mastitis in the postpartum period.

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