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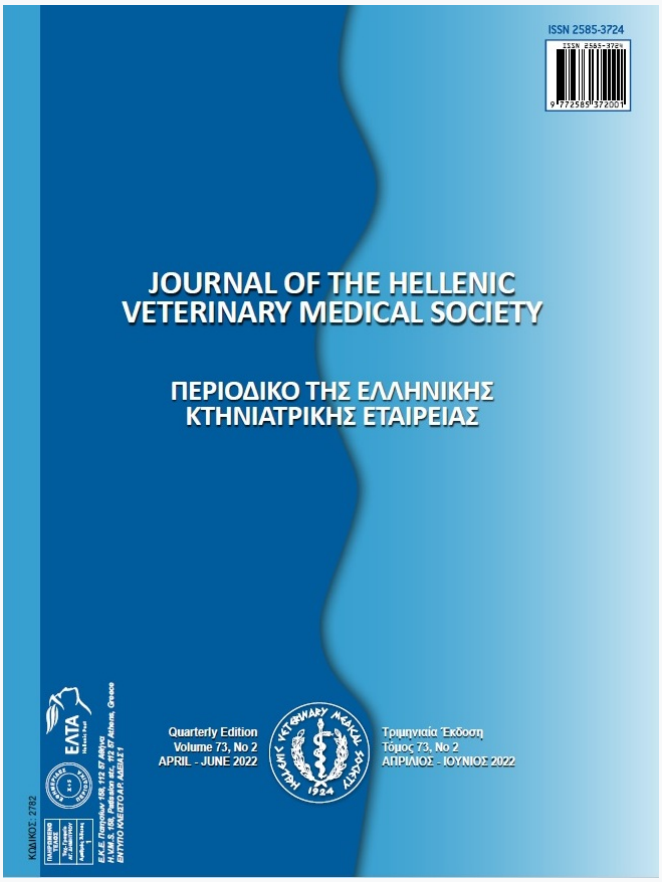

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Savvas Sougaris, CN Brozos, E Petridou, Th. Papadopoulos, E Kiossis

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Abrupt and gradual drying-off procedure and intramammary dry treatment: Impact on udder health status of Chios breed dairy sheep

S. Sougaris[a], C. N. Brozos[a], E. Petridou[b], Th. Papadopoulos[b], E. Kiossis[a]

[a] *Clinic of Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*

[b] *Faculty of Veterinary Medicine, Laboratory of Microbiology and Infectious Diseases, Aristotle University of Thessaloniki, Thessaloniki, Greece*

ABSTRACT: In order to evaluate the impact of drying-off procedure on udder health status and the effect of intramammary dry treatment on prevention of new intramammary infections and improving cure rate of mammary abnormalities, a randomized single blinded controlled trial was performed in which 80 Chios breed dairy ewes were randomly allocated into 2 groups. Ewes of Group A (n=40) dried off gradually during a 15-day period, whereas in ewes of Group B (n=40), udder drying-off took place abruptly. Half of the ewes of each group received intramammary infusion (1 syringe/teat) of benzathinecloxacilline (IDT subgroup, n=20). The rest of the ewes of each group received no treatment at all (control subgroup, n=20). Representative samples of teat duct material and milk were aseptically collected from each mammary half for cytological and microbiological examination. Samples were collected using both conventional and aseptic techniques at the time of enrollment until the final milking before dry off (7 to 14 days before the expected dry period), at dry period (approximately 65 days) and continued at lambing until the end of the ongoing milking period.61.8% of bacterial isolates obtained from teat duct and milk were identified as coagulase-negative staphylococci (CNS).No significant differences were noticed between the two groups in the frequency of mammary gland infection peri-partum (p=0.466), in the risk of new mammary infections during dry period (p=0.750) and in the cure rate of any subsequent mammary infection (p=0.131).Drying-off procedure had no significant impact on somatic cell counts (p=0.760) or milk leucocyte subpopulations (p>0.05) but had a significant effect on milk production of the next lactation period (p<0.001). Ewes treated with antibiotic agent presented a significantly higher cure rate of subsequent mammary infections (p=0.036) and a significantly lower risk of new mammary infections (p=0.039) during dry period, compared to the control group.No statistically significant differences were noticed concerning the impact of treatment on cytological profiles (p>0.5), somatic cell counts (p=0.581) and milk production (p=0.705). The results strengthen the hypothesis that drying off procedure does not affect cure rate neither reduce the risk of new infections during dry period and has no effect on development of mastitis around dry period whereas the use of intramammary dry treatment provides a better bacteriological cure rate of the mammary gland and decreases the possibility of new intramammary infections during dry period.

Keywords: milk cessation method; subclinical mastitis; CNS; dry ewes antibiotic; sheep

Corresponding Author:
S. Sougaris, 94, Vas. Olgas avenue, 54643, Thessaloniki, Greece
E-mail address: ssoungar@icloud.com

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INTRODUCTION

Mastitis and mammary infections have an important economic effect on dairy ewes' farming. Affected glands frequently suffer partial or complete damage and cannot resume their normal secretory function (Mørk et al., 2007). This can cause severe milk production losses and lead to decreased growth and reduced performance of nursed lambs (Chaffer et al., 2003). Additional losses, associated with the cost of treatment and the premature culling, deteriorate the financial impact of intramammary infections (Mørk et al., 2007).

Mastitis in dairy small ruminants is predominantly subclinical (mean prevalence is about 10-37%), whereas clinical incidence is often less than 5% (Al-Majali and Jawabreh, 2003; Gebrewahid et al., 2012; Kiossis et al., 2013, 2007). Unlike cows, the most commonly isolated microorganisms during mastitis in sheep are various coagulase-negative staphylococci (CNS) (prevalence ranges between 25-93%) which elicit high somatic cell count (SCC) and cause important losses of milk yield (Gonzalo et al., 2004; Kiossis et al., 2013).

Dry period (DP) is a time of milking pause before lambing which is necessary for the udder of every milking ruminant and has an impact in the next lactating period (Church et al., 2008; Pinedo et al., 2011; Bradley et al., 2015).

Despite the fact that the importance of dry period with respect to udder health, productivity, mastitis control and prevention and overall health in the next lactation period has been well documented (Bergonier and Berthelot, 2003; Petridis and Fthenakis, 2014), the involution period, following dry off, is associated with an increased susceptibility to new intramammary infections (IMIs) mainly due to the milking stop and the slow transition to a non-secretory state. IMIs just before or right after dry off can affect lactogenesis and impair milk production (Sordillo et al., 1987; Oliver and Sordillo, 1989; Bradley and Green, 2004; Fthenakis et al., 2012;).

Transition into dry period ("dry off") can be achieved either gradually or abruptly (Petridis et al., 2013; Petridis and Fthenakis, 2014; Gelasakis et al., 2015; Zobel et al., 2015). In gradual cessation of lactation (or intermittent milking), milking frequency is progressively reduced over a period of days or weeks until eventually terminates beyond a lower threshold of milk production, although the duration until dry off could vary. Abrupt cessation of lactation can occur by terminating normal daily milking at a particular day, typically determined by the expected lambing.

Additionally, intramammary dry treatment (IDT), at the end of lactation, is one of the most effective strategies to cure any IMIs present at drying-off and to prevent new IMIs in the early dry period (Gonzalo et al., 2004; Linage and Gonzalo, 2008). IDT has the advantage of administration when animals are not being milked so there is no milk discard or antibiotic contamination of bulk tank milk (Mavrogianni et al., 2011; Baştan et al., 2015). Although IDT has been reported to provide cure rates that range between 61.3 and 95.8% (De Santis et al., 2001; Chaffer et al., 2003; Shwimmer et al., 2008; Kiossis et al., 2013), yet the use of such treatment, especially as a prophylactic strategy, is controversial because of the increasing concerns about antimicrobial resistance and its effects on human health. Development of additional or alternative management practices that decrease the risk of IMI's around DP would be beneficial in dairy sheep industry (Landers et al., 2012; Gott et al., 2016).

Few studies have evaluated the effect of drying off procedure and the use of IDT on IMIs and udder bacteriological health status (Petridis et al., 2013; Petridis and Fthenakis, 2014) but none of them, conducted on dairy sheep, have investigated the impact of cessation method or IDT on milk yield and quality (SCC and leukocyte subpopulations) in the subsequent lactation.

The primary objective of this study was to evaluate the importance of drying off procedure (gradual or abrupt) in relation with IDT on subsequent mammary infection, development of mastitis peri-partum and prevention of new IMIs during DP. The secondary objective was to investigate its possible effects on milk yield, SCC and differentiation of somatic cell subpopulations during the forthcoming lactation period.

MATERIALS AND METHODS

Flock and animal selection

For the study, a Chios breed dairy ewes flock, located in Central Macedonia area, was selected on the basis of relatively high milk production and convenience during study protocol.

After a thorough clinical examination, emphasizing on the mammary gland and SCC of all the eligible ewes of the flock, a total of 80 of them were randomly chosen to enroll on the study for over a 12-month

period. The eligible milking ewes were at the age of 3.66 ± 0.95 years, of similar bodyweight and were found free of any clinical or subclinical mammary gland infection (two functional udder halves, no clinical abnormalities, macroscopically normal milk, SCC <400x10^3/ml, bacteriological counts <400 cfu/ml) (Las Heras et al., 1999).

Experimental design

During the experiment, all animals were housed in a barn with sheltered and open area (approximately 1.0-1.2 m^2/ewe) until the end of lactation. Enrolled ewes were randomly assigned into two groups depending on the drying off procedure, following simple randomization procedures (such as computer-generated randomization list). Ewes of Group A (n=40) dried gradually during the last two weeks of lactation; animals of this group were milked once daily for the first 7 days of this period and every other day for the rest 7 days of lactation. Animals of Group B (n=40) followed the farm's regular milking schedule until the last day of their lactation period and were dried-off by abrupt cessation of milking. At the day of dry off (D_0), mean milk production per ewe was similar in both groups (0.327 lt and 0.407 lt for Group A and Group B, respectively, *p=0.235*). Animals of both groups where mechanically milked.

At the final milking just before dry off (D_0), half of the animals of each group (n=20) received an intramammary infusion of a commercially available dry cow product containing 500mg bezanthine cloxacilline (Orbenin DC®, Zoetis), whereas the rest of the ewes received no treatment at all and served as controls. The researchers were responsible for the proper administration of intramammary dry treatment.

Dry ewes remained into DP for approximately 65 days in a separate sheltered barn (approximately 1.0-1.3 m^2/ewe), where no other systemic or topical treatment was performed. Right before lambing, pregnant ewes were transferred into lambing pens (approximately 1.5 m^2/ewe and 0.1 m^2/lamb, additionally) where they stayed until the end of lambing.

Sampling for both groups performed at the period just before dry off (P_0-D_0), during the dry period (D_1-D_3) and at lambing until the end of the next lactation period (L_0-L_8).

Specifically, for Group A (gradual drying-off, n=20) sampling started 11 days before dry period (P_0) and were carried out every other day (6 samplings in total). Samplings for Group B (abrupt drying-off, n=20) initiated 4 days before dry period and continued every other day (3 samplings in total). At the last sampling before dry off (D_0), intramammary dry treatment was administered right after sample collection and the animals inserted into the dry period. Additional samplings performed at days 5, 10 and 20 into the dry period (D_1-D_3), for both groups.

Sampling initiated again at lambing (L_0) taking care of collecting samples before the newborn lambs sucked their dam's udder for the first time and continued atday 5 after lambing (L_1) as well as at the weaning which took place at day 60 post-partum (p.p.) for both groups (L_2).

Finally, further samplings were performed at the upcoming milking period, every 30 days, for a total of 6 samplings (L_3-L_8).

Sampling collection and procedure

All ewes were distinctively marked with ear tags in order that researchers and farm personnel could easily determine which group the ewes belong to, at every particular timestamp.

At every sampling occasion, daily milk production (MP) for every ewe was recorded from farm's electronic archives. Animals were placed into the slots of the milking pen until the end of sampling.

Each udder half's skin was thoroughly cleaned and disinfected using Chlorexidine glyconate 4% w/v while povidone iodine solution was applied at teat skin. Continuously, teat and udder were washed using alcohol solution and dried with sterile gauzes (Fthenakis, 1994; Mavrogianni et al., 2006).

Teat duct material sampling was carried out by a modification of the technique proposed by Mavrogianni et al., 2006, using a sterile thin metallic swab with transportation tube (80.1363 Amisw/och., Sarstedt AG&Co. KG, Germany, Sarstedtstraße 1, Nümbrecht, 51588) instead of an intravascular catheter. The 5mm cotton tip of the swab was inserted, under aseptic conditions, into the teat duct and scrubbed on the mucosa of teat canal and orifice, by rolling movement. Once the swab was removed, it was placed back to the transportation material, taking care not to contaminate the sample.

To collect milk samples, the first 2-3 streams of secretion were discarded into a dark container in order to check for any abnormal findings. Continuously,

5-10 ml of secretion were aseptically obtained into a sterile sample tube and maintained accordingly until the end of sampling (Ariznabarreta et al., 2002; Fthenakis et al., 2012).

Additional samples of secretion were obtained for somatic cells differentiation (Gonzalo et al., 2003, 1998).

Finally, two milk samples were taken to perform SCC and to evaluate milk chemical composition of each udder half, respectively.

In every sampling occasion, all samples were collected by the researchers from both udder halves and teat duct of every ewe of the experimental flock.

At the end of each sampling, both milk and teat duct material samples were forwarded to the laboratory for further analysis within 2 hours using icebox.

Sample analysis and laboratory examination

Milk samples obtained for somatic cells differentiation were properly prepared, coated onto film smears and dyed after May-Grünwald-Giemsa stain (Gonzalo et al., 2003, 1998); in each film smear, 100 cells were counted, differentiated and recorded by the same investigator.

Counting of somatic cells was performed using an automatic DNA-particle counting device (Fossomatic FC, N. Foss Electric, Hillerød, Denmark) whereas milk chemical composition was analysed by a fully automated infrared milk analyser (Milkoscan FT 6000, N. FossElectric, Hillerød, Denmark) for each milk sample.

Concerning the microbiological examination, each teat duct swab as well as 100μl of each mastitic milk sample were spread separately onto sheep blood agar, mannitol salt agar and McConkey agar plates (OXOID-Germany). All plates were incubated aerobically at 37^{o}C for 48h.The mannitol positive colonies were encountered as *Staphylococcus aureus* while mannitol negative as Coagulase-Negative Staphylococci. The result multiplied then by 10 in order to assess the subclinical mastitis using the limit of 400cfu/ml. Five (5) suspected as *Staphylococcus spp*. colonies of each plate were subjected to oxidase and catalase test as well as to coagulase production test. All positive to both catalase and oxidase tests strains were then identified at the species level using API Staph (bioMérieux) biochemical panel. Lactose positive colonies were biochemically identified using API 20E (bioMérieux) panel.Moreover, all CNS isolates were subjected to pulsed field gel electrophoresis (PFGE) in order to identify the presence of the same or different clones of bacteria.

Data analysis

In the present study, due to the number of the sampling times and the long intervals between them, there was a difficulty in precisely estimating whether an udder half kept being infected between two consecutive samplings or healed in the meantime and reinfected just before the next sampling occasion. Therefore, the calculation of some values (i.e. incident rate, cure rate) was almost impossible. For this reason, several assumptions had been made, according to Petridis et al. (2012): i) when isolation of pathogenic bacteria occurred from teat duct material or milk then this considered to be a teat duct/mammary gland infection, accordingly; ii) an udder half or teat duct could be either infected or uninfected at every given moment; iii) if a teat duct or mammary gland, at a particular time, considered to be uninfected consequently it was at risk of new infection whereas an infected one was no longer at risk; iv) if a teat duct/mammary gland was infected by the same pathogen in two consecutive sampling times, it was considered to be infected throughout the time between these two sampling times; on the other hand, an uninfected teat duct/mammary gland in two consecutive sampling occasions, considered to be uninfected in the meantime, as well; v) if a teat duct/mammary gland was infected in one sampling time but found to be uninfected in the next one then the infection considered to be eliminated in the meantime of the two sampling times; similarly, teat ducts/mammary glands uninfected in one sampling time but infected on the next one indicated that the infection occurred in between of the two sampling occasions.

Definitions

Cure rate (or *cure fraction*) defined as the proportion of udder halves and/or teat ducts that were infected just before DP (P_0-D_0) but presented no signs of infection (“cured”) right after lambing (L_0-L_2). *Incidence rate* (or *new infection rate*) defined as the proportion of new infections of udder halves and/or teat ducts that were at risk of infection during dry period. These rates calculated by the formulas described by Petridis et al. (2013). *Frequency* of infection defined as the total number of mammary and/or teat duct infection cases before (P_0-D_0) and after dry period (L_0-L_2).

Statistical analysis

All statistical analyses were conducted using the statistical language R (R Core Team, 2013) and the generalized linear mixed effects models. Specifically, in order to study the effect of drying-off method and IDT on cure rate, frequency and incident rate of mammary infection and on the mean values of MP, SCC and somatic cell subpopulations, Generalized Linear Mixed Effects (GLME) modeling was used (Pinheiro and Bates, 2000). The optimal fixed component structure, which provides information about the explanatory factors affecting the mean values of dependent variables, was defined through the methodology proposed by Zuur et al (2009). Describing briefly, a full model with all the main and interaction effects was initially specified and compered to the nested model after dropping out insignificant terms through the likelihood ration (LR) test. Moreover, the initial model incorporated a control independent variable, describing whether a subject diagnosed as infected at each timestamp of the experimental setup. The aim of this insertion into the examined model was to control the variation caused from infected subjects during the study period and study potential interaction effects with the other examined factors. Finally, graphical validation was used to assess the underlying assumptions of homogeneity and normality of residuals of the selected models.

Specifically, for the SCC measurements, fitting of the models on the raw SCC values revealed departures from the homoscedasticity and normality assumptions, so logarithmic transformation of the raw measurements (logSCC) was used to normalize the distribution of SCC.

In all tests a difference was considered as statistically significant when p-value (significance) was less than 0.05. All the tests conducted were two-tailed (non-directional) in the sense that the alternative hypothesis is that the measures tested are not equal.

A power analysis was conducted using the G*Power software. Milk yield was selected as the output of choice of the experiment in power analysis and the results indicate a power of 92.9%. The 92.9% of statistical power indicates that, with a sample size of 80 ewes, the probability to reject a true alternative hypothesis is only 7.1%, which is very low, and such probability is considered sufficient for the application of the statistical test.

RESULTS

During this research, 7 animals died post-partum (4 animals from Group A, 3 in the treated lot and 1 in controls, and 3 animals from Group B, 2 in the treated lot and 1 in controls) from causes irrelevant to the study while lambing was performed at the appropriate lambing pens under normal conditions.

Amongst bacterial isolations, CNS was the predominant species (61.8%); *S. aureus*, *E. coli*, *Klebsiella* spp, *Pseudomonas sp* were isolated as well. Detailed results are shown in Table 1.In 14 out of 32 cases of both mammary gland and teat duct CNS infection of the same subject (udder half), the exact same CNS clone isolated from both milk and teat duct material samples, whereas, in the rest of the cases, infection caused by different CNS clones.

Concerning drying off procedure, both groups presented no significant differences peripartum as far as intramammary infection frequency is concerned (p=0.466) (Table 2).

No significant differences were noticed between the two groups in bacteriological cure rate (p=0.131) of intramammary infection, as well. Additionally, no significant differences were observed in the risk of new infections of teat duct or mammary gland (p=0.750) during dry period between the two groups (Table 3).Furthermore, there was no statistically significant interaction between milk leukocyte subpop-

Table 1: Total bacterial isolates obtained from teat duct and milk samples from udder halves of ewes of both groups (gradual and abrupt drying-off, with and without the use of intramammary dry treatment) throughout the study

	Milk	Teat duct	Total (%)
CNS	60	34	94 (61.8)
Staph. aureus	10	9	19 (12.5)
E. coli	6	6	12 (7.9)
Streptococcus spp	7	2	9 (5.9)
Pseudomonas spp	9	4	13 (8.6)
Klebsiella spp	4	1	5 (3.3)
Total	96	56	152

Table 2: Frequency of mammary infection in udder halves of ewes, in which drying-off took place gradually (Group A, n=80) or abruptly (Group B, n=80), with or without the use of intramammary dry treatment (IDT or control subgroup, accordingly)

	Gradual		Abrupt	
	IDT	Controls	IDT	Controls
P_0-D_0	0.293		0.311	
L_0-L_2	0.206	0.368	0.250	0.368

D_0: Day of the start of dry period, L_0: Day of the subsequent lambing.
Same superscript in the same row indicates statistically significant difference at *P<0.05*

Table 3: Cure rate and incident rate of mammary infection in udder halves of ewes, in which drying-off took place gradually (Group A, n=72) or abruptly (Group B, n=74)

	Group A	Group B	*p*
Cure rate	0.250	0.625	0.131
Incident rate	0.083	0.068	0.750

Level of significance: *P<0.05*

Table 4: Mean proportions of leucocyte subpopulations in milk samples from ewes, in which drying-off took place gradually (Group A, n=40) or abruptly (Group B, n=40)

	Group A				Group B			
	M	L	PMNL	E	M	L	PMNL	E
D_0	48.71	8.59	37.92	4.78	49.19	8.34	38.13	4.34
D_1-D_3	45.53	7.74	41.73	5	51.37	6.99	37.47	4.19
L_0-L_2	39.38	6.63	48.33	5.66	38.49	7.86	49	4.65
L_3-L_8	45.90	6.73	41.72	5.65	48.38	7.46	39.72	4.44

D_0: Day of the start of dry period, D_1-D_3: Dry period samplings, L_0-L_2: Day of the subsequent lambing until weaning, L_3-L_8: Lactation samplings
M: Macrophages, L: Lymphocytes, PMNL: Polymorphonuclear cells, E: Epithelial cells
Same superscript in the same row indicates statistically significant difference at *P<0.05*

Table 5: Somatic cell counts (logSCC) in udder halves of ewes, in which drying-off took place gradually (Group A, n=80) or abruptly (Group B, n=80), with or without the use of intramammary dry treatment (IDT or controls subgroup, accordingly)

	Group A		Group B	
	IDT	Controls	IDT	Controls
D_0	242.00		127.50	
L_0-L_2	152.94	199.32	165.81	572.53
L_3-L_8	601.10	683.78	241.09	749.45

D_0: Day of the start of dry period, L_0-L_2: Day of the subsequent lambing until weaning, L_3-L_8: Lactation samplings
Same superscript in the same row indicates statistically significant difference at *P<0.05*

Table 6: Milk production of ewes, in which drying-off took place gradually (Group A, n=40) or abruptly (Group B, n=40), with or without the use of intramammary dry treatment (IDT or controls subgroup, accordingly)

	Group A		Group B	
	IDT	Controls	IDT	Controls
L_0-L_2	0.83^a	0.73^b	1.23^a	1.29^b
L_3-L_8	0.97^a	0.95^b	1.32^a	1.23^b

L_0-L_2: Day of the subsequent lambing until weaning, L_3-L_8: Lactation samplings
Same superscript in the same row indicates statistically significant difference at *P<0.05*

Table 7: Cure rate and incident rate of mammary infection in udder halves of ewes that received intramammary dry treatment (n=70) compared to the controls (n=76)

	IDT	Controls	*P*
Cure rate	0.667	0.143	0.036*
Incident rate	0.029	0.118	0.039*

Level of significance: *P<0.05*

Table 8: Mean proportions of leucocyte subpopulations in milk samples between ewes that received intramammary dry treatment and controls (IDT and control subgroups, respectively)

	IDT (n=35)				Controls (n=38)			
	M	L	PMNL	E	M	L	PMNL	E
L_0-L_8	48.55	7.06	38.83	5.56	46.87	7.05	41.41	4.67

L_0: Day of the subsequent lambing
M: Macrophages, L: Lymphocytes, PMNL: Polymorphonuclear cells, E: Epithelial cells
Same superscript in the same row indicates statistically significant difference at *P<0.05*

Table 9: Mean proportions of leucocyte subpopulations in milk samples from infected and uninfected ewes' udder halves, respectively

	Infected				Uninfected			
	M	L	PMNL	E	M	L	PMNL	E
P_0-D_0	31.54[a]	5.54[b]	60.05[c]	2.87[d]	46.11[a]	7.32[b]	40.14[c]	6.43[d]
L_0-L_8	34.88[a]	5.96	55.54[b]	3.62	47.71[a]	7.05	40.12[b]	5.11

P_0: Day of the start of drying-off procedure, D_0: Day of the start of dry period, L_0: Day of the subsequent lambing
M: Macrophages, L: Lymphocytes, PMNL: Polymorphonuclear cells, E: Epithelial cells
Same superscript in the same row indicates statistically significant difference at *P<0.05*

ulations and drying-off procedure (Table 4). Finally, both groups presented no statistically significant differences (p=0.760) concerning logSCC (Table 5).

On the other hand, a statistically significant main effect of drying-off procedure on milk production after lambing was noticed (p<0.001), suggesting that ewes belonging to the group of gradual drying-off procedure presented generally lower milk production on the next milking period (Table 6).

As far as intramammary dry treatment is concerned, new infection rate of teat duct or mammary gland infection is significantly lower in ewes that received antibiotic treatment compared to controls (p=0.039).A statistically significant interaction between intramammary dry treatment and cure rate was noted amongst the two groups (p=0.036); ewes that received intramammary dry treatment presented better bacteriological cure rates opposed to the controls (Table 7).Regarding the frequency of teat duct or mammary gland infection, no statistically significant differences were found (p=0.495) between the animals that received intramammary dry treatment and the controls (Table 2). No significant differences were observed on the impact of intramammary dry treatment on SCC, milk production and leukocyte subpopulations on either group (Table 5, Table 6 and Table 8). Finally, significant differences were found in leukocyte subpopulation proportions between infected and uninfected mammary glands (Table 9).

DISCUSSION

Transition to dry period poses a potential risk of new intramammary infections albeit its necessity for every dairy ruminant. Through this period, the mammary gland is shifting towards a cytological and immunological preparation for the forthcoming milking period. Dry period can also become either an effective barrier in developing new intramammary infections or can restrain underlying infection of previous lactation period (Bergonier and Berthelot, 2003; Fthenakis et al., 2012; Petridis et al., 2012; Hernandez et al., 2015).

Entrance into dry period can be done either gradually or abruptly and can be assisted by the administration of an intramammary antibiotic agent.

Risk-benefit balance should be considered when choosing the appropriate method of drying-off mammary. Abrupt cessation of milking can lead to accumulation of milk into the mammary gland cistern causing increased intramammary pressure and milk leakage from teat apex, thus allowing bacteria to penetrate the teat canal and colonize the gland. Also, the increased volume of milk contains lower concentrations of natural protective factors (i.e. lactoferrin, immunoglobulins and phagocytic cells). In gradual cessation, on the other hand, removal of milk serves to flush bacteria from the mammary gland and may increase the concentration of protective non-specific defense substances in milk but also, it may contribute in increasing the risk of infection of the mammary gland during milking (Dingwell et al., 2001; Petridis et al., 2013).

The initial target of the present study was to estimate whether each of these strategies has a significant impact on udder health during dry period. The results demonstrate that drying-off procedure does not sig-

nificantly affect udder health status by minimizing the risk of new infections of teat duct or mammary gland or preventing the development of subsequent mastitis, as no differences were noted in frequency of teat duct or mammary infection, bacteriological cure rate and risk of new infections between the two methods. Similar results were reported by Petridis et al. (2013) using smaller number of Lacaune crossbreed ewes (19 and 12 ewes in the group of progressive and abrupt drying-off, respectively).

Gradual and abrupt drying-off procedures were equivalent in regard to their effect on SCC in subsequent lactation, as well (Table 5). There are no previous data in dairy ewes, although our results are in accordance with those of Gott et al. (2017) who reported that milk cessation method in dairy cows was not significantly associated with SCC in subsequent lactation.

Additionally, no difference was observed in the cytological profiles of leukocyte subpopulations, between the two study groups at the day of dry off and after lambing (Table 4). Similarly, Petridis et al. (2013) found that there was no association between milk cessation method and the mean leukocyte subpopulation after the subsequent lambing.

Conversely, as far as the impact of drying-off procedure on milk production of the next milking period is concerned, we found that there is a significant difference between the two methods (Table 6). Ewes that inserted into DP gradually produced significantly lower milk production at the subsequent lactation than those of the abrupt drying-off group. Although there are no previous data in dairy sheep, our results do not come in agreement with those conducted in dairy cows. Gott et al. (2017) reported no significant differences in milk production of dairy cows between gradual and abrupt drying-off procedure.

On the other hand, intramammary dry therapy is a crucial factor in udder health management. In the present study, ewes that received antimicrobial preparation prior to dry period presented a significantly lower risk of being infected during this period than the controls. Chaffer et al. (2003) reported lower new infection rates in treated groups (15.6%) opposed to control groups (28.6%), although the differences were not significant. Furthermore, in a study of Linage and Gonzalo (2008), new infection rates were found to be lower to the treated compared to the control lots (7.9% and 22.8%, respectively) supporting the preventive effect of dry treatment against intramammary infections during dry period.

Apart from being an important preventive barrier, the administration of IDT leads to a better bacteriological cure rate, as well; in the present study, ewes that received IDT presented to be significantly more effective in overcoming any underlying mammary than the controls. Similar results by DeSantis et al. (2001) demonstrated significant differences in bacteriological cure rate between treated (61.3-82.3%) and control lots (33.3-48.1%). In another study, Chaffer et al. (2003) noticed that animals treated with a combination of procaine benzylpenicilline, nafcilline and dihydrostreptomycin presented better bacteriological cure rates (52.6-64.9%) than those of animals with no treatment at all (6.5%). Furthermore, Linage and Gonzalo (2008) observed that groups of ewes received IDT presented better cure rates compared to control lots, whereas, Shwimmer et al. (2008b) reported a decrease of bacterial infection in the treated lots.

On the contrary, our results demonstrate that IDT has not significantly affected neither milk production nor SCC, on uninfected udder halves. Cytological profiles in ewes' milk can be affected by different stages of lactation (Paape et al., 2001; Shah et al., 2017) and can be altered by intramammary infections during certain periods (Albenzio et al., 2002; Paape et al., 2007), as presented in Table 8 but there is no impact of drying-off method or IDT on mean proportions of leukocyte subpopulations in healthy ewes.

In the present study, the most prevalent isolated pathogens were CNS (62.8% in milk and 60.7% in teat duct) followed by *Staphylococcus aureus* (10.4% in milk and 16.07% in teat duct). The high prevalence of infections caused by CNS (25-93%) can be attributed to poor farm management, farm hygiene or milking management practices (Gelasakis et al., 2015). Isolation of bacteria at lambing, before the newborn lambs suck their dam's udder for the first time, indicates that infection happened into the dry period. Especially for CNS, it has been reported that certain species could survive host defense mechanisms or antibiotic administrations and persist throughout dry period or it is possible to re-infect mammary gland multiple times. This is probably due to the presence of certain CNS in the microflora of teat skin or environment of the animals (Kiossis et al., 2013) and under certain conditions can colonize teat apex and infect mammary gland via teat canal. Our results reveal that in 14 out of 32 cases of mammary and teat duct infection, the exact clone of

bacteria isolated supporting the hypothesis that colonization of teat duct could lead to mammary infection, nevertheless, further research has to be conducted.

Although the benefits of antibiotic dry treatment are indisputable, however, concerns about the uncontrolled use of antibiotic agents and the antibiotic resistance have questioned the need of "complete" instead of "selective" dry therapy in dairy ewes. Despite the difficulties and the cost of determination of the animals that need to receive IDT at dry-off, selective dry treatment contributes to the decrease of the number of animals that require treatment, so the use of antibiotic agents is being rationalized reducing the risk of antibiotic resistance and antibiotic residues in the food chain (Bergonier and Berthelot, 2003; Petridis and Fthenakis, 2014). It would be of great interest that studies will be conducted towards selective dry treatment and its interaction with the different drying off procedures.

Moreover, intramammary dry treatment in dairy small ruminants is well documented to be one of the most effective practices for mastitis control during dry period (Bergonier and Berthelot, 2003; Contreras et al., 2007), however, in meat small ruminants the effectiveness of this preventive measure remains controversial (Pereira et al., 2018). In meat sheep and goats, dry period could be as long as 6 months (ranging from 5 to 10 months) and it is considered as a limiting factor to the use of intramammary dry treatment, since the majority of the drugs available could not remain active throughout this long dry period (Pereira et al., 2018). Additionally, spontaneous cure rates in meat small ruminants could range from 20-67% (Bergonier and Berthelot, 2003; Contreras et al., 2007; Spanu et al., 2011), leading to an effective self-healing process, regardless the administration of intramammary dry treatment (Fox et al., 1992). Nevertheless, more studies have to be conducted in order to precisely evaluate the use of this preventive procedure in meat small ruminants.

CONCLUSION

The result of this study suggests that drying off procedure has no effect on the development of mastitis or subsequent mammary infection. Mammary involution can be performed either gradually or abruptly without being affected by the duration or the pattern of the procedure used. However, the antibiotic dry therapy of Chios breed dairy ewes prior to dry period provides a better bacteriological cure rate and improves udder health by decreasing the risk of new intramammary infections during this period.

CONFLICT OF INTEREST

The authors of the present study have nothing to disclose.

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