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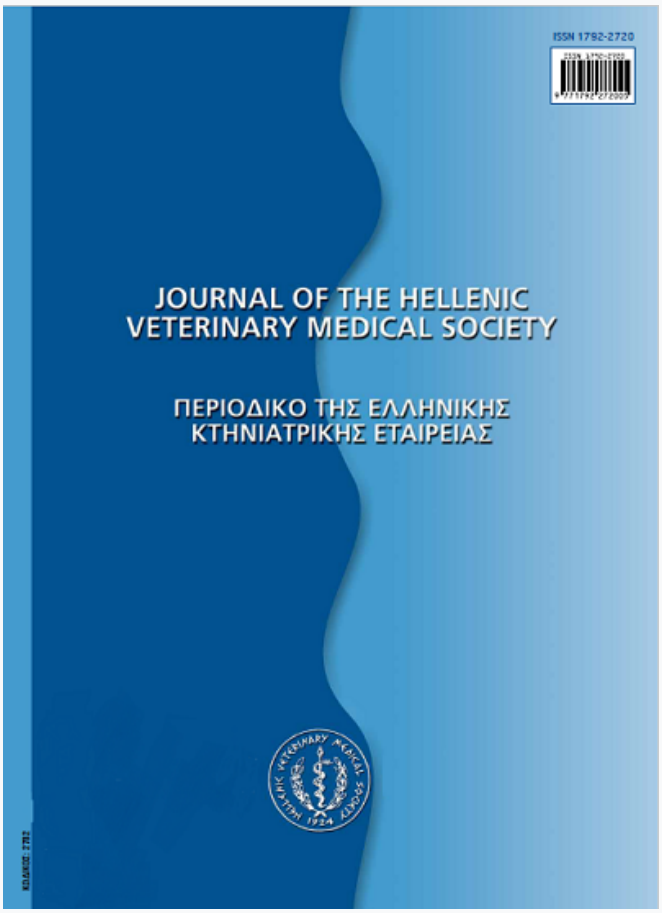

Effects of drying-off procedure of ewes' udder, with intramammary antibiotic administration, in subsequent mammary infection and development of mastitis

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Effects of drying-off procedure of ewes' udder, with intramammary antibiotic administration, in subsequent mammary infection and development of mastitis

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Επίδραση της διαδικασίας ξήρανσης του μαστού σε προβατίνες, με ενδομαστική χορήγηση αντιβιοτικών, στην επακόλουθη μόλυνση αυτού και την εκδήλωση μαστίτιδας

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ABSTRACT. Objective of the study was to evaluate effects of the procedure followed for drying-off of ewes' udder in subsequent mammary infection and development of mastitis, in an experiment, where intramammary antibiotic administration (procaine penicillin and neomycin) was performed into the right mammary gland of animals at end of lactation period. In ewes of group A, drying-off took place progressively during a period of 22 days; in ewes of group B, drying-off took place abruptly. Samples of teat duct material and milk for bacteriological and cytological examination were collected before start of the drying-off procedure and on two occasions after the subsequent lambing. Median time to first teat duct infection *post-partum* was 2 and 4.5 days (left and right, respectively) for group A and 6.5 and 3.5 days for group B ($P > 0.38$); median time to first mammary infection *post-partum* was 4.5 and 7 days (left and right, respectively) for group A and 6.5 and 3.5 days for group B ($P > 0.22$). Principal bacterial isolates were coagulase-negative staphylococci. No significant differences were observed between the two groups in *post-partum* frequency of: teat duct infection ($P > 0.17$), mammary infection ($P > 0.36$), subclinical mastitis ($P > 0.36$), abnormal findings in a mammary gland ($P > 0.17$). No significant differences were seen between the two groups in *post-partum* incidence risk of the following outcomes: teat duct infection ($P > 0.75$), mammary infection ($P > 0.42$), subclinical mastitis ($P > 0.39$), abnormal findings in a mammary gland ($P > 0.85$). No significant differences were evident between the two groups in cure rate of abnormal findings in a mammary gland ($P > 0.89$); a significant difference was evident between left and right mammary glands ($P < 0.045$). The results support a hypothesis that the procedure for udder drying-off (i.e., progressive or abrupt cessation of lactation) does not appear to affect the risk

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of subsequent mammary infection and development of mastitis, in cases of intramammary administration of antibiotics at the end of a lactation period. Intramammary administration of antibiotics improved cure rates of mammary abnormalities, independently of the procedure followed for udder drying-off.

Keywords: dry-ewe mastitis, mammary infection, mammary involution, sheep

ΠΕΡΙΛΗΨΗ. Η μελέτη αποσκοπούσε στην αξιολόγηση της επίδρασης της διαδικασίας ξήρανσης του μαστού σε προβατίνες, στην επακόλουθη μόλυνση των μαστικών αδένων και την εκδήλωση μαστίτιδας σε έναν πειραματισμό, στον οποίο πραγματοποιήθηκε ενδομαστική χορήγηση αντιβιοτικών στο τέλος της γαλακτικής περιόδου. Στα ζώα της ομάδας Α (n=6), η ξήρανση του μαστού πραγματοποιήθηκε προοδευτικά, σε διάστημα 22 ημερών, ενώ στα ζώα της ομάδας Β (n=6), η ξήρανση του μαστού πραγματοποιήθηκε απότομα. Στο τέλος της γαλακτικής περιόδου, πραγματοποιήθηκε ενδομαστική χορήγηση συνδυασμού προκαϊνικής πενικιλλίνης και νεομυκίνης στο δεξιό μαστικό αδένα όλων των ζώων (ομάδες Α και Β). Συλλέχθηκαν δείγματα υλικού θηλαίου πόρου και γάλακτος για βακτηριολογική και κυτταρολογική εξέταση πριν την έναρξη της διαδικασίας ξήρανσης του μαστού, καθώς δύο φορές μετά τον τοκετό των ζώων: το πρώτο δείγμα συλλέχθηκε μέχρι την 4η ημέρα και το δεύτερο από την 5η μέχρι τη 10η ημέρα μετά τον τοκετό. Η διάμεση τιμή του διαστήματος για την πρώτη μόλυνση μετά τον τοκετό ήταν 2 και 4,5 ημέρες για τους θηλαίους πόρους (αριστερούς και δεξιούς, αντίστοιχα) και 4,5 και 7 ημέρες για τους μαστικούς αδένες (αριστερούς και δεξιούς, αντίστοιχα) για την ομάδα Α. Για την ομάδα Β, τα αντίστοιχα διαστήματα ήταν 6,5 και 3,5 ημέρες για τους θηλαίους πόρους και τους μαστικούς αδένες (αριστερούς και δεξιούς, αντίστοιχα) (για όλες τις συγκρίσεις, $P > 0,22$). Από τα 38 βακτηριακά στελέχη που απομονώθηκαν, 74% ήταν πηκτάση-αρνητικοί σταφυλόκοκκοι. Μετά τον τοκετό, δεν παρατηρήθηκαν σημαντικές διαφορές μεταξύ των δύο ομάδων στη συχνότητα μόλυνσης του θηλαίου πόρου ($P > 0,17$), μόλυνσης του μαστικού αδένα ($P > 0,36$), υποκλινικής μαστίτιδας ($P > 0,36$) ή παθολογικών καταστάσεων στο μαστό ($P > 0,17$). Επίσης, δεν παρατηρήθηκαν σημαντικές διαφορές μεταξύ των δύο ομάδων στο ποσοστό προσβολής για καμία παράμετρο που μελετήθηκε: μόλυνση του θηλαίου πόρου ($P > 0,75$), μόλυνση του μαστικού αδένα ($P > 0,42$), υποκλινική μαστίτιδα ($P > 0,39$) ή παθολογικές καταστάσεις στο μαστό ($P > 0,85$). Τέλος, δεν παρατηρήθηκαν σημαντικές διαφορές μεταξύ των δύο ομάδων στο ποσοστό θεραπείας των παθολογικών καταστάσεων στο μαστό ($P > 0,89$) μεταξύ των ομάδων Α και Β, παρατηρήθηκε όμως σημαντική διαφορά μεταξύ αριστερών και δεξιών μαστικών αδένων ($P < 0,045$). Τα ευρήματα υποστηρίζουν την υπόθεση ότι η διαδικασία ξήρανσης του μαστού των προβατίνων (δηλαδή, προοδευτική ή απότομη ξήρανση) δεν επηρεάζει την πιθανότητα μόλυνσης αυτού και την εκδήλωση μαστίτιδας, σε περιπτώσεις ενδομαστικής χορήγησης αντιβιοτικών στο τέλος της γαλακτικής περιόδου. Η ενδομαστική χορήγηση αντιβιοτικών στο τέλος της γαλακτικής περιόδου βελτίωσε το ποσοστό θεραπείας των παθολογικών καταστάσεων στο μαστό, ανεξάρτητα από τη διαδικασία ξήρανσης που έλαβε χώρα.

Λέξεις ευρετηρίασης: μαστίτιδα ξηρής περιόδου, μόλυνση του μαστικού αδένα, παλινδρόμηση του μαστικού αδένα, πρόβατα

INTRODUCTION

Mammary involution is the process of regression of mammary tissue to a non-secreting state, with disappearance of much of the epithelial tissue, and takes place after cessation of lactation. In sheep, mammary involution may be progressive (or gradual), which occurs at the end of a lactation period as milk production declines, or abrupt (or induced or initiated), which is the result of stopping milk removal (Hurley, 1989), because milking is terminated or lambs are removed; senile involution, which occurs at the end of the reproductive life of an animal, is rarely observed, as ewes would usually be culled earlier as a result of health management practices in a flock.

We have already reported results, which did not support a hypothesis that the procedure followed for udder drying-off (i.e., abrupt or progressive cessation of lactation) would affect the risk of infection of the mammary glands during the dry period and the immediately *post-partum* period (Petridis et al., 2011). However, reports studying the problem with simultaneous administration of antibiotics at the end of a lactation period have not been published. Objective of the present work was to evaluate effects of the procedure followed for drying-off of ewes' udder in subsequent mammary infection and development of mastitis, tested in an experiment, where intramammary antibiotic administration was performed at the end of a lactation period.

MATERIALS AND METHODS

Experimental design

The experiment was carried out in a semi-intensive dairy flock in Central Greece. Lambs were removed from their dams at the age of 45 to 60 days and, then, ewes in the flock were hand-milked twice daily. The experiment started after a lactation period 6 to 8 month-long.

In total, 12 Lacaune-cross multiparous ewes were used. Animals were allocated at random in one of two groups. In ewes of group A (n=6), drying-off took place progressively during a period of 22 days; initially, ewes were hand-milked once daily for a week (D1-D7), which was followed by another week during which ewes were hand-milked once every two days (D9, D11, D13), followed by a final week during which ewes were hand-milked once every three days (D16, D19, D22); in total, during the whole process, ewes were hand-milked on 12 occasions, always by the same milker. In ewes of group B (n=6), drying-off took place abruptly; ewes were milked on D0 for the last time and no milking was carried out after that.

On D22 for group A animals and on D0 for group B animals (i.e., at the end of that lactation period), intramammary administration of a combination of 500 mg procaine penicillin and 300 neomycin sulphate (NEO-MASTITAR; MSD Animal Health, Boxmeer, The Netherlands) was carried out into the right mammary gland of all animals (A and B).

Subsequently, animals were mated. During pregnancy, no animal received any antimicrobial agents. At the end of pregnancy, ewes were moved to individual pens, where they lambed normally 183 to 209 days after D0 (day of lambing: L0). Teat duct material and milk samples were collected again, on two occasions *post-partum*.

Samplings and examinations performed

At the start of the drying-off procedure (on D0), all ewes were clinically examined, with special attention paid to their mammary glands and teats (Fthenakis, 1994; Saratsis et al., 1998; Mavrogianni et al., 2005). A thorough disinfection was carried out using povidone iodine scrub solution on the teat apex and the lower (1 cm) part of the teat skin. A fine (20 G), plastic, sterile catheter (Abbocath®, Abbott) was used for sampling the teat duct and collecting teat duct material. The stylet was taken out and the catheter was cut with a sterile blade to a length of 2 mm. In order to ensure accurate and consistent cutting of the catheter at the desired length, a sterilized ruler was always placed beside the catheter. The whole procedure was carried out under aseptic conditions. The investigator (IGP) held the catheter from the cannula hub; it was inserted into the teat, rolled around the internal teat wall, in order to sample the mucosa, and then withdrawn. Description and validation details of the method have been presented previously (Mavrogianni et al., 2006). Milk samples were then obtained. The first two squirts of secretion were drawn onto the palm of the gloved hand of the investigator and examined for the presence of abnormal signs; then, 10 to 15 ml of secretion were carefully collected into a sterile container.

In all cases, samples were collected from both teats and both mammary glands of each ewe. Initially, samples were obtained on D0 before start of the drying-off procedure [‘I’ samples]. Subsequently to lambing (L0), further samples were collected. The first sample was collected on L0 to L4 [‘II’ samples] and the second sample on L5 to L10 [‘III’ samples]; in all cases, an interval of ≥5 days elapsed between the two *post-partum* sampling occasions.

Samples of material collected on the tip of the catheter (‘teat duct material’) and milk samples were plated onto Columbia 5% sheep blood agar; the media were incubated aerobically at 37 oC for up to 72 h. Throughout this study, all bacteria isolated were identified by using conventional techniques (Barrow and Feltham, 1993; Euzeby, 1997).

The California Mastitis Test (CMT) was carried out in milk samples, as described by Fthenakis (1995) for ewes’ milk. Five degrees of reaction scores were recognized; reactions scored ≥‘1’ were considered to be indicative of increased cellular content in milk. Leucocyte subpopulations were identified by direct microscopy after Giemsa stain of milk films; in each case 100 cells were observed and counted.

Data management and analysis

In the present study, there is a difficulty with attempts at estimating incidence rate (new ‘infection’ per individual at risk for each time point at risk). In many cases, an individual teat duct / mammary gland might change from being ‘infected’ to being ‘unin-

fected' and *vice-versa*; therefore, when there was a long time-interval between sampling occasions, it was not possible to know what happened between the two sampling occasions, i.e. how many infections and 'cures' there might have been. Therefore, the following definitions were initially made: (i) 'isolation of bacteria' was equivalent to 'infection with'; 'isolation of bacteria from the teat duct material' was equivalent to 'infection of the teat duct' and 'isolation of bacteria from the milk' was equivalent to 'infection of the mammary gland'; (ii) on a particular sampling point, a teat duct / mammary gland was defined as being 'at risk of becoming infected' (i.e., yielding bacteria during the microbiological examination) if it had been uninfected (i.e., did not yield any bacteria during the microbiological examination) on the previous sampling point; (iii) on the subsequent sampling point, this teat duct / mammary gland could be either 'infected' (in which case it was not at risk) or 'uninfected' (in which case it was still at risk); (iv) on subsequent sampling occasions, if this teat duct / mammary gland was 'uninfected', then it was again 'at risk'; (v) if a teat duct / mammary gland was infected on one sampling occasion but not on the next one, then the infection was deemed to have been eliminated half-way between the two sampling occasions; conversely, if a teat duct / mammary gland was uninfected on one sampling occasion and infected on the next one, then the infection was considered to have taken place half way between the two occasions; (vi) if a teat duct / mammary gland was infected with the same organism on two consecutive sampling occasions, then it was infected throughout the time between those two sampling occasions; conversely, if a teat duct / mammary gland was uninfected on two consecutive sampling occasions then it was uninfected throughout the time between those two sampling occasions.

Based on the above, it was possible to calculate an estimate of the length of time a teat or a mammary gland was at risk before it became infected; teats ducts / mammary glands contributed more than one value if they became uninfected and then were re-infected. These results were modelled by using survival analysis.

Analysis of results was carried out by comparing changes in status between 'I' *versus* 'II' and 'III' samples. Incidence rate was calculated from the formula: M/[A-(V/2), where M: number of new cases during the *post-partum* period (i.e., either on L0-L4 or on L5-L10), A: animals at risk at lambing and V: number of animals withdrawn from the study during the internal time period evaluated (Martin et al., 1987). Cure rate was calculated from the formula: N/[B-(W/2), where N: number of cases that had shown the outcome of interest on D0, but did not show it again during the *post-partum* period (i.e., neither on L0-L4, nor on L5-L10), B: number of cases that had shown the outcome of interest on D0 and W: number of animals withdrawn from the study during the internal time period evaluated (Martin et al., 1987).

During the analysis, the following outcomes were evaluated: 'teat duct infection' (i.e., isolation of bacteria from teat duct material samples), 'mammary infection' (i.e., isolation of bacteria from milk samples), 'subclinical mastitis' (i.e., isolation of bacteria from a milk sample coupled with increased CMT score in the same sample, but with no clinically detectable abnormalities in that mammary gland) and 'abnormal findings in a mammary gland' (i.e., presence of mammary abnormalities, defined as any case of subclinical mastitis or of clinically detectable abnormal findings, including clinical mastitis, in a mammary gland). Specifically for cure rate, the only outcome evaluated was 'abnormal findings in a mammary gland'.

In all cases, teat duct material and milk samples were considered separately, as were the left (untreated control) and the right (antibiotic-treated) side of the udder. Statistical significance was assessed by the Sign Test, which allowed for the readings to be paired. Data were modelled in Minitab 14 (Minitab Inc., State College, PA, USA).

Significance level was set at $P = 0.05$, on a 2-sided null hypothesis of no difference.

RESULTS

All animals lambed normally. No lamb deaths were recorded during the study.

Median time to first teat duct infection *post-partum* was 2 and 4.5 days (left and right, respectively) for group A and 6.5 and 3.5 (left and right, respectively) for group B ($P > 0.38$). Median time to first mammary infection *post-partum* was 4.5 and 7 days (left and right, respectively) for group A and 6.5 and 3.5 (left and right, respectively) for group B. In all comparisons, no significant differences were seen ($P > 0.22$). Bacteria were isolated always in pure culture. Of the 38 bacterial isolates obtained, 74% were coagulase-negative *Staphylococcus*

spp.; moreover, *Bacillus* spp., *Escherichia coli, Mannhemia haemolytica and Staphylococcus aureus* were also isolated. Detailed results are in Table 1.

No significant differences were observed between A and B groups in the *post-partum* frequency of teat duct infection (P>0.17), of mammary infection (P >0.36 for both left and right mammary glands), of subclinical mastitis (P = 1.00 for left and P > 0.36 for right mammary glands) or of abnormal findings in a mammary gland (P>0.36 for left and P >0.17 for right mammary glands). Moreover, no significant differences were observed between left and right side of the

Table 1. Frequency of bacterial isolates obtained from teat duct material and milk samples from ewes, in which udder drying-off took place progressively (group A) or abruptly (group B), with intramammary antibiotic administration in their right mammary gland at cessation of lactation.

	Group A (n=6)	Group B (n=6)
Isolates from teat duct material samples		
Samples 'I'-L (collected on D0)	*S. caprae*: 1 *S. epidermidis*: 1 *S. simulans*: 1	*S. xylosus*: 1
Samples 'I'-R (collected on D0)	*Bacillus* spp.: 1 *Staphylococcus caprae*: 1 *S. epidermidis*: 1 *S. simulans*: 1	*S. chromogenes*: 1 *S. epidermidis*: 1 *S. xylosus*: 1
Samples 'II'-L (collected on L0-L4)	*M. haemolytica*: 1 *S. chromogenes*: 1	
Samples 'II'-R (collected on L0-L4)	*S. aureus*: 1	*S. epidermidis*: 1
Samples 'III'-L (collected on L5-L10)	*M. haemolytica*: 1 *S. epidermidis*: 1	*E. coli*: 1
Samples 'III'-R (collected on L5-L10)	*M. haemolytica*: 1	*S. epidermidis*: 2
Isolates from milk samples		
Samples 'I'-L (collected on D0)	*S. caprae*: 1 *S. epidermidis*: 1 *S. simulans*: 1	*S. xylosus*: 1
Samples 'I'-R (collected on D0)	*S. caprae*: 1 *S. epidermidis*: 1 *S. simulans*: 1	*S. chromogenes*: 1 *S. epidermidis*: 1
Samples 'II'-L (collected on L0-L4)	*M. haemolytica*: 1	
Samples 'II'-R (collected on L0-L4)		*S. epidermidis*: 1
Samples 'III'-L (collected on L5-L10)	*M. haemolytica*: 1 *S. epidermidis*: 1	*E. coli*: 1
Samples 'III'-R (collected on L5-L10)	*M. haemolytica*: 1	*S. epidermidis*: 2

D0: day of start of the drying-off procedure, L0: day of subsequent lambing.
L: left, R: right.

Table 2. Frequency of teat duct infection, of mammary infection, of subclinical mastitis and of abnormal findings in a mammary gland in ewes, in which udder drying-off took place progressively (group A) or abruptly (group B), with intramammary antibiotic administration in their right mammary gland at cessation of lactation, during the first 10 days *post-partum*.

	Group A (n=6)	Group B (n=6)
Teat duct infection		
Samples 'I'-L (collected on D0)	0.500	0.167
Samples 'I'-R (collected on D0)	0.667	0.600
Samples 'II'-L (collected on L0-L4)	0.333	0.167
Samples 'II'-R (collected on L0-L4)	0.000	0.167
Samples 'III'-L (collected on L5-L10)	0.333	0.167
Samples 'III'-R (collected on L5-L10)	0.167	0.333
Mammary infection		
Samples 'I'-L (collected on D0)	0.500	0.167
Samples 'I'-R (collected on D0)	0.500	0.400
Samples 'II'-L (collected on L0-L4)	0.167	0.000
Samples 'II'-R (collected on L0-L4)	0.000	0.167
Samples 'III'-L (collected on L5-L10)	0.333	0.167
Samples 'III'-R (collected on L5-L10)	0.167	0.333
Subclinical mastitis		
Samples 'I'-L (collected on D0)	0.333	0.000
Samples 'I'-R (collected on D0)	0.333	0.200
Samples 'II'-L (collected on L0-L4)	0.000	0.000
Samples 'II'-R (collected on L0-L4)	0.000	0.167
Samples 'III'-L (collected on L5-L10)	0.167	0.167
Samples 'III'-R (collected on L5-L10)	0.167	0.000
Abnormal findings in a mammary gland		
Samples 'I'-L (collected on D0)	0.333	0.500
Samples 'I'-R (collected on D0)	0.333	0.833
Samples 'II'-L (collected on L0-L4)	0.333	0.167
Samples 'II'-R (collected on L0-L4)	0.000	0.333
Samples 'III'-L (collected on L5-L10)	0.500	0.500
Samples 'III'-R (collected on L5-L10)	0.167	0.333

D0: day of start of the drying-off procedure, L0: day of subsequent lambing.
L: left, R: right.
'Teat duct infection' = isolation of bacteria from teat duct material samples; 'mammary infection' = isolation of bacteria from milk samples; 'subclinical mastitis' = isolation of bacteria from a milk sample coupled with increased CMT score in the same sample; 'abnormal findings in a mammary gland' = presence of mammary abnormalities, defined as any case of subclinical mastitis or of clinically detectable abnormal findings in a mammary gland, including clinical mastitis.
Same superscript in the same row or in the same column indicates statistically significant difference at $P < 0.05$.

Table 3. Incidence rate of teat duct infections, of mammary infections, of subclinical mastitis and of abnormal findings in a mammary gland in ewes, in which udder drying-off took place progressively (group A) or abruptly (group B), with intramammary antibiotic administration in their right mammary gland at cessation of lactation, during the first 10 days *post-partum*.

	Group A (n=6)	Group B (n=6)
Teat duct infection		
L	0.333	0.200
R	0.500	0.500
Mammary infection		
L	0.000	0.200
R	0.000	0.333
Subclinical mastitis		
L	0.000	0.167
R	0.000	0.250
Abnormal findings in a mammary gland		
L	0.250	0.333
R	0.000	0.000

L: left, R: right.
'Mammary infection' = isolation of bacteria from milk samples; 'subclinical mastitis' = isolation of bacteria from a milk sample coupled with increased CMT score in the same sample; 'abnormal findings in a mammary gland' = presence of mammary abnormalities, defined as any case of subclinical mastitis or of clinically detectable abnormal findings in a mammary gland, including clinical mastitis.
Same superscript in the same row or in the same column indicates statistically significant difference at $P < 0.05$.

udder in frequency of teat duct infection (P>0.2), of mammary infection (P>0.36), of subclinical mastitis (P > 0.2) and of abnormal findings in a mammary gland (P > 0.17) for both groups. Detailed results are in Table 2.

No significant differences were seen between A and B groups in the *post-partum* incidence risk of any of the outcomes studied: teat duct infection (P = 0.754 for left and P = 1.00 for right mammary glands), mammary infection (P = 0.753 for left and P = 0.423 for right mammary glands), subclinical mastitis (P = 0.623 for left and P = 0.391 for right mammary glands) or abnormal findings in a mammary gland (P = 0.851 for left and P = 0.852 for right mammary glands). Moreover, no significant differences were seen between left and right side of the udder in any of the outcomes studied: teat duct infection (P > 0.67), mammary infection (P > 0.75), subclinical mastitis (P> 0.79) and abnormal findings in a mammary gland (P> 0.39), for both groups. Detailed results are in Table 3.

Finally, no significant differences were evident between A and B groups in cure rate (during mammary involution and the first 10 days *post-partum*) of abnormal findings in a mammary gland (P = 0.956 for left and P = 0.887 for right mammary glands). A significant difference was evident between left and right mammary glands (P = 0.027 and P = 0.043 for group A and B, respectively). Detailed results are in Table 4.

No significant differences were evident in leucocyte subpopulations between groups A and B after lambing. Detailed results are in Table 5.

DISCUSSION

In sheep, effective udder health management at the end of a lactation period is important for maintaining mammary health during the subsequent lactation period. However, potential interactions of mechanisms involved in mammary involution with mammary health have not been studied in ewes as extensively as in cows.

The procedure of drying-off is part of the udder health management of ewes. The aims of effective udder

Table 4. Cure rate of abnormal findings in a mammary gland in ewes, in which udder drying-off took place progressively (group A) or abruptly (group B), with intramammary antibiotic administration in their right mammary gland at cessation of lactation, during mammary involution and the first 10 days *post-partum*.

	Group A (n=6)	Group B (n=6)
L	0.000[a]	0.333[b]
R	0.500[a]	0.600[b]

L: left, R: right.
'Abnormal findings in a mammary gland' = presence of mammary abnormalities, defined as any case of subclinical mastitis or of clinically detectable abnormal findings in a mammary gland, including clinical mastitis.
Same superscript in the same row or in the same column indicates statistically significant difference at $P < 0.05$.

Table 5. Mean proportion of leucocytes in milk samples from ewes, in which udder drying-off took place progressively (group A) or abruptly (group B), with intramammary antibiotic administration in their right mammary gland at cessation of lactation.

	Group A (n=6)	Group B (n=6)
Abnormal findings in a mammary gland		
Samples 'I'-L (collected on D0)	Lym: 11%, Mac: 9%, Neu: 80%	Lym: 26%, Mac: 25%, Neu: 49%
Samples 'I'-R (collected on D0)	Lym: 9%, Mac: 10%, Neu: 81%	Lym: 33%, Mac: 45%, Neu: 22%
Samples 'II'-L (collected on L0-L4)	Lym: 8%, Mac: 17%, Neu: 75%	(2)
Samples 'II'-R (collected on L0-L4)	(1)	Lym: 5%, Mac: 11%, Neu: 84%
Samples 'III'-L (collected on L5-L10)	Lym: 10%, Mac: 20%, Neu: 70%	Lym: 6%, Mac: 20%, Neu: 74%
Samples 'III'-R (collected on L5-L10)	Lym: 3%, Mac: 7%, Neu: 90%	Lym: 6%, Mac: 20%, Neu: 74%
No abnormal findings in a mammary gland		
Samples 'I'-L (collected on D0)	Lym: 20%, Mac: 29%, Neu: 51%	Lym: 7%, Mac: 34%, Neu: 59%
Samples 'I'-R (collected on D0)	Lym: 21%, Mac: 13%, Neu: 66%	Lym: 9%, Mac: 10%, Neu: 81%
Samples 'II'-L (collected on L0-L4)	Lym: 5%, Mac: 19%, Neu: 76%	Lym: 7%, Mac: 27%, Neu: 66%
Samples 'II'-R (collected on L0-L4)	Lym: 10%, Mac: 25%, Neu: 65%	Lym: 8%, Mac: 27%, Neu: 65%
Samples 'III'-L (collected on L5-L10)	Lym: 8%, Mac: 27%, Neu: 65%	Lym: 10%, Mac: 19%, Neu: 71%
Samples 'III'-R (collected on L5-L10)	Lym: 10%, Mac: 33%, Neu: 57%	Lym: 10%, Mac: 27%, Neu: 63%

D0: day of start of the drying-off procedure, L0: day of subsequent lambing.
L: left, R: right.
Lym: lymphocytes, Mac: macrophages, Neu: neutrophils.
(1): no samples into that category at that sampling point, (2): sample not possible to process.

health management at the end of a lactation period are (i) to cure infections which have occurred during the previous lactation and (ii) to prevent development of new mammary infections during the dry period (Fthenakis et al., 2012). Frequently, udder health management would include intramammary administration of antibiotics (Mavrogianni et al., 2011), which has been documented to have a beneficial effect to decreasing bulk somatic cell counts in dairy flocks (Hueston et al., 1989; Chaffer et al., 2003; Gonzalo et al., 2004; Scwimmer et al., 2008; Spanu et al., 2011); the procedure may be just as useful in ewes in mutton-type production systems (Hendy et al., 1981). The present results also indicated that, after intramammary administration of antibiotics at the end of a lactation period, improved cure rates of mammary abnormalities were recorded, independently of the procedure followed for udder drying-off.

In mutton-type production systems, cessation of lactation, and consequent mammary involution, is abrupt, taking place when lambs are removed from their dam (Sargison, 2008). In dairy-type production systems, cessation of lactation can be effected either progressively (i.e. milking frequency can be gradually decreased over a period of several days or weeks) or abruptly (i.e. milking is stopped) (Gelasakis et al., 2010).

One may postulate that the method followed for drying-off the mammary glands could affect their health status (Newman et al., 2009). For example, one may suggest that accumulation of milk into the mammary gland cistern may predispose to bacterial multiplication and subsequent development of mastitis, whilst removal of milk (even at less frequent than usual intervals) serves to flush bacteria from the mammary gland; moreover, milk accumulation into the mammary cistern leads to increased intramammary pressure, which can cause leakage of milk from the teat and delayed formation of the protective keratin plug within the teat duct (Dingwell et al., 2004; Odensten et al., 2007). In contrast, periodic (albeit infrequent) removal of milk may contribute to increasing the risk of infection of the mammary gland, by means of increased risk of mammary infection during milking, whilst, on the other hand, it may increase concentration of protective non-specific defence substances (e.g., lactoferrin, IgG) accumulated in the secretion of the involuting gland, thus making the mammary gland more resistant to infections (Newman et al., 2009). Finally, during accumulation of milk into the mammary gland, the defence substances and the leucocytes can contribute to effective bacterial killing (Lee and Outteridge, 1981). Although numbers of animals used in the study were small, the present results allied to those of Petridis et al. (2011; 2013) confirm that the method of udder drying-off does not affect the development of the risk of subsequent mammary infection and development of mastitis subsequently to the next lambing. The beneficial effects of intramammary antibiotic administration confirm previous results in reducing the rate of infection (Fthenakis et al., 2012).

CONCLUDING REMARKS

The results support a hypothesis that the procedure for udder drying-off (i.e., progressive or abrupt cessation of lactation) does not appear to affect the risk of subsequent mammary infection and development of mastitis, in cases of intramammary administration of antibiotics at the end of a lactation period. Intramammary administration of antibiotics improved cure rates of mammary abnormalities, independently of the procedure followed for udder drying-off.

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