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How does the milk removal method affect teat tissue and teat recovery in dairy ewes?

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The aim of this work was to study how machine milking (MM) carried out in suitable conditions affects teat wall thickness and teat canal length and their return after milking to pre-milking conditions, in comparison with other milk extraction methods considered biological referents: lamb suckling (LS), milk removal by catheter (RC) and hand milking (HM). Three Latin square experiments were designed, using 11 ewes in the first experiment (LS), 13 in the second (RC) and 12 in the third (HM). Each of the Latin squares was divided into two periods: in the first, the left gland of each animal was machine milked and the corresponding treatment (LS, RC and HM) was applied to the right gland. Subsequently, in the second period the extraction methods were interchanged. During the experimental period, 4 sampling days were carried out (2 in each experimental period), where ultrasound scans were taken before (B) and immediately after milking (A) and at 1 (1 h), 2 (2 h), 3 (3 h), 4 (4 h), 6 (6 h), 8 (8 h) and 10 (10 h) hours after milking finished. Teat wall thickness (TWT), teat wall area (TWA), teat end area (TEWA) and teat canal length (TCL) were measured in all the ultrasound images. MM increased TWT after milking compared with RC. TWT, TWA, TEWA and TCL were lower ($P<0{\cdot}05$) in HM than in MM. No significant differences ($P>0{\cdot}05$) were found between LS and MM for any variable. The extraction method affected the recovery time of the variables, with total teat recovery at 6 h after RC and 4 h after HM. In the case of LS, the TEWA and TCL values were recovered sooner, as of 3 h. Teat recovery time after MM was similar to the extraction method with which it was compared in each experiment. Thus, considering the similar increase in wall thickness and their recovery time compared with the reference methods, it was concluded that machine milking, carried out in optimum conditions and respecting the time interval between milkings usually applied on sheep farms (8–12 h), would not affect teat integrity. Moreover, given the variability observed in teat thickness recovery time between the different experiments, further research should be carried out to study which factors intrinsic and extrinsic to the animal may affect the teat wall thickness and recovery time after machine milking.

Keywords: Recovery teat, ewe, congestion, ultrasonography, machine milking.

Calf suckling, milk extraction by catheter and hand milking are considered physiological methods of reference in cattle to assess physiological alterations of the teat caused by machine milking (Hamann & Burvenich, 1994) as these methods either cause no changes in teat tissues or any such changes are minimal (Gleeson et al. 2002), and the teat wall thickness when measured by cutimeter may even be diminished (Hamann & Mein, 1988).

In cattle, suckling, milk removal by catheter and hand milking give rise to lower teat tissue thickness values than machine milking (Hamann & Mein, 1990; Gleeson et al. 2002), as also occurs in dairy goats (Alejandro et al. 2014a). These differences are attributed to the conditions in which the milk is extracted by each method. In machine milking, during the massage phase the liner collapses on the teat end, generating a compressive force that closes the teat canal, stopping milk flow and re-evacuating the liquid accumulated in the teat end. Despite this, the teat end is still exposed to milking vacuum (van der Tol et al. 2010) which does not occur during offspring suckling. During suckling, only the teat end is exposed to a high vacuum, as the rest of the teat is protected and supported by the tongue and palate (van der Tol et al. 2010). In milk extraction by catheter

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and hand milking, no vacuum is applied to the teat; in the former case, the milk drains from the udder owing to the difference between glandular and atmospheric pressure (Hamann & Mein, 1988) and in manual milking the milk is forced through the teat canal by high intracisternal pressure that builds up when the teat is compressed by hand. The amount of pressure built up within the teat sinus is highly variable and depends on the force applied by hand and the patency of the teat canal (McDonald & Witzel, 1966).

After conventional mechanical milking, the teat needs some hours to recover its pre-milking status and during this period the penetrability of microorganisms into the teat channel may increase, thus exacerbating the risk of new intramammary infections (Hamann & Osteras, 1994). In practical situations, teat tissue changes are the most suitable method to estimate teat canal penetrability (Neijenhuis et al. 2001). Hamann & Mein (1990) noted that TWT measured by cutimeter returns to pre-milking values after 15 min in calf suckling and in less than 1 h in manual milking, although this time is prolonged up to 4 h in teats milked with modified milking equipment (pulsation only at the teat base and pulse chamber pressure 35 kPa over atmospheric pressure). In bovine cattle, TWT gauged by ultrasound is recovered at around 4 h after milking (Neijenhuis et al. 2001; Gleeson et al. 2002) whereas the teat canal and teat end thickness need more than 8 h (Neijenhuis et al. 2001). In goats, the recovery time is greater than 10 h, both in machine milking (MM) and in other reference methods (Alejandro et al. 2014a). In sheep, recovery time after MM may exceed 10 h (Wójtowski et al. 2006).

Owing to the limited information available on the effect of different milk extraction systems on teat status and recovery time in Manchega ewes, the aim of this work was to study how MM affects teat wall thickness and teat canal length and their recovery after milking in normal physiological conditions, compared with other milk extraction methods considered referents: lamb suckling (LS), milk removal by catheter (RC) and hand milking (HM).

Materials and methods

Farm and animal management

The experiment was carried out at the Small Ruminant Educational and Research farm at the Escuela Politécnica Superior de Orihuela at the Miguel Hernández University, (Orihuela, Spain). The farm flock consisted of 80 Manchega breed ewes. The milking parlour was 'Casse' type with quit exit stall, a platform with 12 places and 12 low-line milking units (1 × 12 × 12). Teatcups had an automatic vacuum cut-off valve and silicone liners (Top Flow S) (Gea Farm Technologies, Bönen, Germany). The milking parameters used in the experiment were 36 kPa vacuum level, 180 pulsation/min and 50% pulsation ratio.

Experimental design

To achieve the objectives, three experiments in Latin square design were carried out.

Experiment 1 (EXP 1): lamb suckling (LS) vs. mechanical milking (MM). Eleven ewes were used (weeks 3 and 4 post partum). One day before the pre-experimental period began (5 d duration) the lambs were separated from the ewes into adjacent housing and then reunited with their mothers in the mornings for suckling from the 2 glands during the pre-experimental period and from one of the 2 glands during the experimental period. During the experimental period, when the gland was emptied the lambs were separated into the adjacent housing again, where they were given the milk extracted from the ewe's other gland using buckets with teats. Lambs had free access to alfalfa hay and water.

The experimental phase began on the 6th day and lasted for 14 d. In the first experimental period (7 d), the left gland of each ewe was machine milked while in the right gland the milk was extracted by the suckling lambs. In the second experimental period (remaining 7 d) the gland group treatments (left and right) were interchanged.

Experiment 2 (EXP 2): milk removal by catheter (RC) vs. mechanical milking (MM). Fifteen ewes were used (weeks 6 and 7 post partum). The pre-experimental stage lasted for 2 d, where extraction of milk from both glands was performed by catheter. During this period, two animals suffered intramammary infection and were removed from the experiment. We also decided to shorten the experimental period to 4 d to reduce the risk of new intramammary infections. In the first experimental period (2 d), the left gland of each ewe was machine milked, whereas milk was extracted from the right gland by force of gravity and catheter. In the second experimental period (remaining 2 d) the gland group treatments (left and right) were interchanged.

For milk extraction from the glands by catheter, the procedure was as follows: first, the teat was cleaned with cotton wool dipped in 70% alcohol. Before introducing the catheter, a more thorough cleansing of the teat tip and sphincter was performed. To lower the risk of infection in the gland, the first streams of foremilk was discarded while the catheter was inserted. To remove the catheter once the milk was extracted, a piece of cotton wool dipped in 70% alcohol was placed over the teat sphincter. The catheter was washed, disinfected and submerged in 70% alcohol before being used again.

Experiment 3 (EXP 3): hand milking (HM) vs. mechanical milking (MM). Twelve ewes were used (weeks 4 and 5 post partum). The pre-experimental stage lasted for 2 d and milk extraction from both glands was performed by hand. The experimental phase began on the 3rd day and lasted for 4 d. In the first experimental period (2 d), the left glands of all ewes were machine milked while the right glands were hand milked. In the second experimental period (remaining 2 d) the gland group treatments (left and right) were interchanged.

Variables measured on sampling days

During the pre-experimental period, samples were taken on 2 d in EXP1 and on 1 d in EXP2 and EXP3, taking ultrasound scans of the teats before (B) and after (A) milking. During the experimental period, in total there were 4 sampling days: two controls on the 2 d prior to treatment switchover and two controls on the last 2 d of the experiment. In these controls, ultrasound explorations were performed before milking (B), after milking (A) and at 1 (1 h), 2 (2 h), 3 (3 h), 4 (4 h), 6 (6 h), 8 (8 h) and 10 (10 h) hours after milking was completed.

Ultrasound scanning was performed by the methodology described by Alejandro et al. (2014b) in sheep. A portable ultrasound unit (Agroscan AL, ECM, Noveko International Inc., France) was used, equipped with a 5-MHz linear probe. For the examination, a transparent plastic recipient filled with water at 37 °C was used. The probe was placed inside a latex bag filled with contact gel and contact gel was applied between the latex bag and the recipient. The images obtained were processed using a software program designed for the purpose by the research team (ECOTEAT®) and the following measurements were studied: teat wall thickness (TWT, mean value of the two walls examined, cm), area of the teat walls (TWA, cm^2), teat end area (TEWA, cm^2) and teat canal length (TCL, cm). Although the teat canal was not appreciable in many of the ultrasound images, the ECOTEAT® program was able to estimate TCL by measuring the distance from the teat tip to the end of the teat cistern (outer and inner end of the teat canal, respectively). Thickness increment values were obtained by means of the following formula: Increment (%): 100 × (after milking− before milking)/before milking.

On each sampling day, milk production and extraction time per gland were recorded. For HM and RC the milk collected was weighed on a scale; for LS the lambs were weighed before and after suckling. For MM, production was recorded on the electronic production meters installed in the milking parlour.

Statistical analysis

The association between the explanatory variables and teat tissue variables was assessed using a linear mixed model procedure (Proc Mixed, SAS Institute Inc, V.9.1. 2002). Outcome variables were TWT, TWA, TEWA and TCL measured before and after milking and their increments. For each experiment (1, 2, 3) the following explanatory variables were included as fixed effects: treatment (TRT, 2 levels, 1: LS, RC or HM, for experiment 1,2, and 3 respectively; 2: MM); Period (PER, 2 levels, 1: first period and 2: second period); Day nested within PER (PDAY, 2 levels, 1: first day; 2: second day); Interaction of PER × TRT was confounded with gland within ewe and consequently not included. Ewe and Gland (2 levels, R: right; L: left) nested to Ewe were considered as random terms.

A similar modelling procedure was used to assess teat recovery in each of the treatments assayed in each experiment. As a result, the final model included TWT, TWA, TEWA and TCL as the outcome variables and the following explanatory variables: Moment (M, 9 levels, B: before, A: after, 1, 2, 3, 4, 6, 8, 10 h after milking); Period (PER, 2 levels, 1: first period and 2: second period); Day nested to PER (PDAY, 2 levels, 1: first day; 2: second day). Ewe and Gland (2 levels, R: right; L: left) nested to Ewe were considered as random terms.

Milk production and milking time were not included as covariables in the final model, as there was no significant effect.

Results

At the start of the experiments, the total milk production from the animals at morning milking was 841 ± 127 ml in EXP1, 573 ± 88 ml in EXP2 and 638 ± 99 ml in EXP3. During the pre-experimental periods of the three experiments, no significant differences ($P<0·05$) were found between the left and right glands in any variable, either before or after milking (data not shown). Means of TWT in the pre-experimental period varied from 0·38 to 0·45 cm and from 0·46 to 0·55 cm before and after milking, respectively. TWA average varied from 1·33 to 1·50 cm^2 and 1·43 to 1·58 cm^2 before and after milking, respectively, and TEWA means varied from 0·89 to 1·21 cm^2 and from 1·07 to 1·22 cm^2 before and after milking, respectively. Finally, the average of TCL varied from 0·84 to 1·06 cm and from 1·00 to 1·12 cm before and after milking, respectively.

Effect of milk extraction method on teat wall thickness and teat canal length

Table 1 shows the value of the variables taken by ultrasound (TWT, TWA, TEWA and TCL) before and after milking and their increments in each of the experiments. In EXP1 (LS), no significant differences were found between LS and MM in any of the variables taken by ultrasound before and after milk extraction, nor in the calculated increase in the variables.

MM caused a rise in TWT ($P<0·01$) compared with RC (EXP 2), although there were no significant differences in any variable when comparing the increase between treatments.

After milk extraction, it was observed that in mechanically milked glands the TWT, TWA, TEWA and TCL variables were significantly higher than in manually milked glands (EXP3). However, these differences were not observed in the increments calculated.

The variations in TWT, TWA, TEWA and TCL in EXP1 and EXP3 after machine milking were similar, whereas in EXP2 the variation of the variables was slightly higher. The TWT increment in the three milk extraction methods of reference

Table 1. Effects of milk removal methods on teat wall thickness and teat canal length variables measured by ultrasonography†

	Lamb suckling	Machine milking	L.S.‡	Removal by catheter	Machine milking	L.S.	Hand milking	Machine milking	L.S.
Before extraction									
Teat wall thickness (TWT), cm	0·38 ± 0·02	0·37 ± 0·02	NS	0·46 ± 0·02	0·48 ± 0·02	NS	0·45 ± 0·02	0·47 ± 0·02	NS
Teat wall area (TWA), cm^2	1·32 ± 0·05	1·29 ± 0·05	NS	1·43 ± 0·04	1·46 ± 0·04	NS	1·50 ± 0·04	1·50 ± 0·04	NS
Teat-end area (TEWA), cm^2	0·91 ± 0·06	0·87 ± 0·06	NS	1·03 ± 0·05	0·98 ± 0·05	NS	1·17 ± 0·06	1·18 ± 0·06	NS
Teat canal length (TCL), cm	0·88 ± 0·03	0·86 ± 0·03	NS	0·92 ± 0·03	0·97 ± 0·03	NS	1·04 ± 0·04	1·06 ± 0·04	NS
After extraction									
Teat wall thickness (TWT), cm	0·49 ± 0·02	0·50 ± 0·02	NS	0·54 ± 0·02	0·58 ± 0·02	**	0·54 ± 0·02	0·57 ± 0·02	*
Teat wall area (TWA), cm^2	1·46 ± 0·06	1·50 ± 0·06	NS	1·54 ± 0·03	1·56 ± 0·03	NS	1·55 ± 0·05	1·62 ± 0·05	***
Teat-end area (TEWA), cm^2	1·09 ± 0·08	1·12 ± 0·08	NS	1·20 ± 0·05	1·25 ± 0·05	NS	1·17 ± 0·06	1·31 ± 0·06	**
Teat canal length (TCL), cm	1·02 ± 0·03	1·05 ± 0·03	NS	1·10 ± 0·03	1·15 ± 0·03	NS	1·07 ± 0·03	1·14 ± 0·03	**
Increment (%)									
Teat wall thickness (TWT)	37·43 ± 8·83	38·95 ± 8·83	NS	21·67 ± 4·40	22·56 ± 4·40	NS	24·66 ± 5·85	26·78 ± 5·85	NS
Teat wall area (TWA)	12·64 ± 3·98	17·83 ± 3·98	NS	8·26 ± 2·10	7·83 ± 2·10	NS	4·70 ± 2·37	8·76 ± 2·37	NS
Teat-end area (TEWA)	23·99 ± 6·91	35·57 ± 6·91	NS	29·53 ± 6·01	26·54 ± 6·01	NS	3·53 ± 4·08	13·54 ± 4·08	NS
Teat canal length (TCL)	19·82 ± 4·22	25·03 ± 4·22	NS	23·64 ± 4·22	19·77 ± 4·22	NS	5·42 ± 2·56	8·45 ± 2·56	NS

† Values are means ± SEM.
‡ L.S.: Significance level.
* $P<0·05$; ** $P<0·01$; *** $P<0·001$.

(LS, RC and HM) was similar, although the increment of TWA, TEWA and TCL was lower in manually milked glands.

Effect of milk extraction method on teat recovery

The time taken for TWT, TWA, TEWA and TCL to recover their pre-milking values varied according to the milk extraction method (Tables 2–4). TWT was recovered at 6 h and 4 h in EXP2 and EXP3, respectively. However, recovery time in EXP1 was more than 10 h.

TWA was recovered at 4 and 2 h with RC (EXP2) and HM (EXP3), although in LS (EXP1) recovery time was more than 10 h. TEWA and TCL recovery times were 3 h (EXP1) and 4 h (EXP2), whereas in EXP3 (HM) the values were similar before and after milk extraction, so recovery was immediate.

TWT and TWA recovery time after mechanical milking was very similar to that observed after the respective reference method in each experiment (Fig. 1). However, TEWA was recovered at 10 h in EXP1, at 6 h in EXP2 and at 2 h in EXP3. In the case of TCL, it was recovered at 8 h in EXP1 and at 2 h in EXP2 and EXP3

In all variables and all extraction methods, the greatest value was obtained after machine milking. TWT began to decrease at 1 h in EXP1 and at 2 h in EXP2 and EXP3. TWA decreased as of 1 h in EXP1 and at 2 h in EXP 2. TEWA and TCL decreased as of 1 h in EXP1 and EXP2. In EXP3, TWA decreased at 1 h, whereas TEWA and TCL values did not change after milk extraction.

Discussion

The changes in the TWT, TWA and TCL were similar between milk extraction by lamb suckling and machine milking. In cattle, Hamann & Mein (1988) report that calf suckling gives rise to lower teat thickness values than mechanical milking when the measurements are taken by cutimeter. However, cows were milked with a higher teat end vacuum than in the present experiment. Alejandro et al. (2014a) in goats, found lower increments of TWT, TWA and TEWA in kid suckling than in machine milking.

Knizkova et al. (2005) noted that the degree of stress suffered by the teat during suckling depends on the age of the offspring and the suckling time. These authors found that calf suckling caused a greater increase in average teat temperature during milk period (20 d postpartum) compared with the colostrum period (5 d), weaning period (16 d) and mechanical milking (42·6 kPa). In the present work, no effect of the lambs' age was observed, as during experimental period 2 the lambs were 7 d older than in period 1, but the PER × TRT interaction had no significant effect on the variables. Although high vacuum levels are generated around the teat end in calf suckling (61 kPa, Rasmussen & Mayntz, 1998), the teat end is only exposed to vacuum for half of the time it is during mechanical milking, as when the calf swallows the milk the vacuum drops to values close to zero (Rasmussen & Mayntz, 1998).

Table 2. Mean values of teat wall thickness, areas and canal length variables measured before and after milking at different hourly intervals in Experiment 1 [lamb suckling (LS) vs. mechanical milking (MM)]

		Time‡								
Treatment	Variables†	Before	After	1 h	2 h	3 h	4 h	6 h	8 h	10 h
LS	TWT	$0·38^{a}\pm0·01$	$0·49^{b}\pm0·01$	$0·49^{bc}\pm0·01$	$0·46^{cd}\pm0·01$	$0·47^{bcd}\pm0·01$	$0·48^{bcd}\pm0·01$	$0·46^{d}\pm0·01$	$0·46^{d}\pm0·01$	$0·46^{d}\pm0·01$
	TWA	$1·32^{a}\pm0·04$	$1·46^{b}\pm0·04$	$1·45^{bc}\pm0·04$	$1·41^{cd}\pm0·04$	$1·41^{cd}\pm0·04$	$1·42^{cd}\pm0·04$	$1·40^{d}\pm0·04$	$1·38^{d}\pm0·04$	$1·39^{d}\pm0·04$
	TEWA	$0·91^{a}\pm0·05$	$1,09^{b}\pm0·05$	$1·02^{bc}\pm0·05$	$0·99^{cd}\pm0·05$	$0·96^{acd}\pm0·05$	$0·98^{acd}\pm0·05$	$0·96^{acd}\pm0·05$	$0·92^{ad}\pm0·05$	$0·90^{a}\pm0·05$
	TCL	$0·88^{a}\pm0·03$	$1·03^{b}\pm0·03$	$0·98^{bc}\pm0·03$	$0·97^{cd}\pm0·03$	$0·93^{ade}\pm0·03$	$0·95^{cde}\pm0·03$	$0·92^{ade}\pm0·03$	$0·90^{ae}\pm0·03$	$0·88^{a}\pm0·03$
MM	TWT	$0·37^{a}\pm0·01$	$0·50^{b}\pm0·01$	$0·46^{c}\pm0·01$	$0·45^{c}\pm0·01$	$0·45^{cd}\pm0·01$	$0·45^{cd}\pm0·01$	$0·44^{cd}\pm0·01$	$0·42^{d}\pm0·01$	$0·43^{d}\pm0·01$
	TWA	$1·29^{a}\pm0·04$	$1·50^{b}\pm0·04$	$1·43^{c}\pm0·04$	$1·43^{c}\pm0·04$	$1·41^{c}\pm0·04$	$1·42^{c}\pm0·04$	$1·41^{c}\pm0·04$	$1·38^{cd}\pm0·04$	$1·36^{d}\pm0·04$
	TEWA	$0·87^{a}\pm0·05$	$1·11^{b}\pm0·05$	$1·01^{c}\pm0·05$	$1·01^{c}\pm0·05$	$1·01^{c}\pm0·05$	$1·00^{cd}\pm0·05$	$0·97^{cd}\pm0·05$	$0·93^{cd}\pm0·05$	$0·89^{a}\pm0·05$
	TCL	$0·86^{a}\pm0·03$	$1·04^{b}\pm0·03$	$0·96^{c}\pm0·03$	$0·96^{c}\pm0·03$	$0·97^{c}\pm0·03$	$0·95^{c}\pm0·03$	$0·92^{cd}\pm0·03$	$0·88^{ad}\pm0·03$	$0·87^{ad}\pm0·03$

† TWT, teat wall thickness (cm); TWA, teat wall area (cm^2); TEWA, teat-end area (cm^2); TCL, teat canal length (cm).
‡ Before: before milking. After: after milking; 1 to 10 h after milking.
$^{a–e}$Means without a common letter within a row indicate significant differences at $P<0·05$.

Table 3. Mean values of teat wall thickness, areas and canal length variables measured before and after milking at different hourly intervals in Experiment 2 [milk removal by catheter (RC) vs. mechanical milking (MM)]

		Time‡								
Treatment	Variables†	Before	After	1 h	2 h	3 h	4 h	6 h	8 h	10 h
RC	TWT	$0·46^{a}\pm0·01$	$0·54^{b}\pm0·01$	$0·54^{b}\pm0·01$	$0·52^{bc}\pm0·01$	$0·50^{cd}\pm0·01$	$0·49^{de}\pm0·01$	$0·46^{a}\pm0·01$	$0·47^{ae}\pm0·01$	$0·48^{ae}\pm0·01$
	TWA	$1·44^{a}\pm0·03$	$1·54^{b}\pm0·03$	$1·52^{bc}\pm0·03$	$1·51^{bcd}\pm0·03$	$1·48^{cde}\pm0·03$	$1·46^{ae}\pm0·03$	$1·45^{ae}\pm0·03$	$1·45^{ae}\pm0·03$	$1·47^{ade}\pm0·03$
	TEWA	$0·98^{a}\pm0·05$	$1·20^{b}\pm0·05$	$1·16^{b}\pm0·05$	$1·08^{c}\pm0·05$	$1·06^{c}\pm0·05$	$1·01^{ac}\pm0·05$	$1·06^{ac}\pm0·05$	$1·03^{ac}\pm0·05$	$1·07^{c}\pm0·05$
	TCL	$0·92^{a}\pm0·03$	$1·10^{b}\pm0·03$	$1·07^{b}\pm0·03$	$0·99^{c}\pm0·03$	$0·98^{c}\pm0·03$	$0·94^{ac}\pm0·03$	$0·99^{c}\pm0·03$	$0·96^{ac}\pm0·03$	$0·98^{c}\pm0·03$
MM	TWT	$0·49^{a}\pm0·01$	$0·58^{b}\pm0·01$	$0·56^{bc}\pm0·01$	$0·55^{cd}\pm0·01$	$0·51^{ef}\pm0·01$	$0·53^{de}\pm0·01$	$0·49^{af}\pm0·01$	$0·49^{af}\pm0·01$	$0·50^{af}\pm0·01$
	TWA	$1·46^{a}\pm0·03$	$1·56^{bc}\pm0·03$	$1·56^{b}\pm0·03$	$1·54^{bc}\pm0·03$	$1·52^{cd}\pm0·03$	$1·52^{bcd}\pm0·03$	$1·49^{ad}\pm0·03$	$1·49^{ad}\pm0·03$	$1·50^{ad}\pm0·03$
	TEWA	$1·03^{a}\pm0·05$	$1·25^{b}\pm0·05$	$1·21^{bc}\pm0·05$	$1·13^{d}\pm0·05$	$1·14^{cd}\pm0·05$	$1·12^{d}\pm0·05$	$1·11^{ad}\pm0·05$	$1·10^{ad}\pm0·05$	$1·12^{d}\pm0·05$
	TCL	$0·98^{a}\pm0·03$	$1·15^{b}\pm0·03$	$1·10^{b}\pm0·03$	$1·03^{ac}\pm0·03$	$1·04^{c}\pm0·03$	$1·03^{ac}\pm0·03$	$1·03^{c}\pm0·03$	$1·01^{ac}\pm0·03$	$1·02^{ac}\pm0·03$

† TWT, teat wall thickness (cm); TWA, teat wall area (cm^2); TEWA, teat-end area (cm^2); TCL, teat canal length (cm).
‡ Before: before milking. After: after milking; 1 to 10 h after milking.
$^{a–e}$Means without a common letter within a row indicate significant differences at $P<0·05$.

Table 4. Mean values of teat wall thickness, areas and canal length variables measured before and after milking at different hourly intervals in Experiment 3 [hand milking (HM) vs. mechanical milking (MM)]

		Time‡								
Treatment	Variables†	Before	After	1 h	2 h	3 h	4 h	6 h	8 h	10 h
HM	TWT	$0·46^{a}±0·02$	$0·55^{b}±0·02$	$0·53^{b}±0·02$	$0·53^{b}±0·02$	$0·49^{c}±0·02$	$0·47^{ac}±0·02$	$0·47^{ac}±0·02$	$0·46^{a}±0·02$	$0·47^{ac}±0·02$
	TWA	$1·50^{ac}±0·03$	$1·55^{b}±0·03$	$1·56^{b}±0·03$	$1·54^{ab}±0·03$	$1·53^{abc}±0·03$	$1·51^{ac}±0·03$	$1·50^{c}±0·03$	$1·51^{ac}±0·03$	$1·52^{abc}±0·03$
	TEWA	$1·17^{ab}±0·05$	$1·17^{ab}±0·05$	$1·21^{b}±0·05$	$1·17^{ab}±0·05$	$1·15^{ab}±0·05$	$1·14^{a}±0·05$	$1·15^{ab}±0·05$	$1·16^{ab}±0·05$	$1·21^{b}±0·05$
	TCL	$1·05^{abc}±0·03$	$1·07^{ab}±0·03$	$1·09^{b}±0·03$	$1·06^{abc}±0·03$	$1·03^{ac}±0·03$	$1·02^{c}±0·03$	$1·03^{ac}±0·03$	$1·04^{ac}±0·03$	$1·07^{ab}±0·03$
MM	TWT	$0·45^{af}±0·02$	$0·56^{b}±0·02$	$0·51^{c}±0·02$	$0·50^{cd}±0·02$	$0·49^{de}±0·02$	$0·47^{aef}±0·02$	$0·45^{af}±0·02$	$0·43^{f}±0·02$	$0·45^{af}±0·02$
	TWA	$1·48^{ae}±0·03$	$1·62^{b}±0·03$	$1·55^{c}±0·03$	$1·53^{cd}±0·03$	$1·52^{cde}±0·03$	$1·52^{cde}±0·03$	$1·50^{ade}±0·03$	$1·47^{a}±0·03$	$1·50^{ade}±0·03$
	TEWA	$1·16^{a}±0·05$	$1·33^{b}±0·05$	$1·24^{c}±0·05$	$1·20^{ac}±0·05$	$1·17^{ac}±0·05$	$1·19^{ac}±0·05$	$1·18^{ac}±0·05$	$1·16^{a}±0·05$	$1·21^{ac}±0·05$
	TCL	$1·05^{ad}±0·03$	$1·15^{b}±0·03$	$1·11^{bc}±0·03$	$1·08^{ac}±0·03$	$1·05^{ad}±0·03$	$1·04^{ad}±0·03$	$1·06^{ad}±0·03$	$1·03^{d}±0·03$	$1·06^{acd}±0·03$

† TWT, teat wall thickness (cm); TWA, teat wall area (cm^2); TEWA, teat-end area (cm^2); TCL, teat canal length (cm).
‡ Before: before milking. After: after milking; 1 to 10 h after milking.
a–e Means without a common letter within a row indicate significant differences at $P<0·05$.

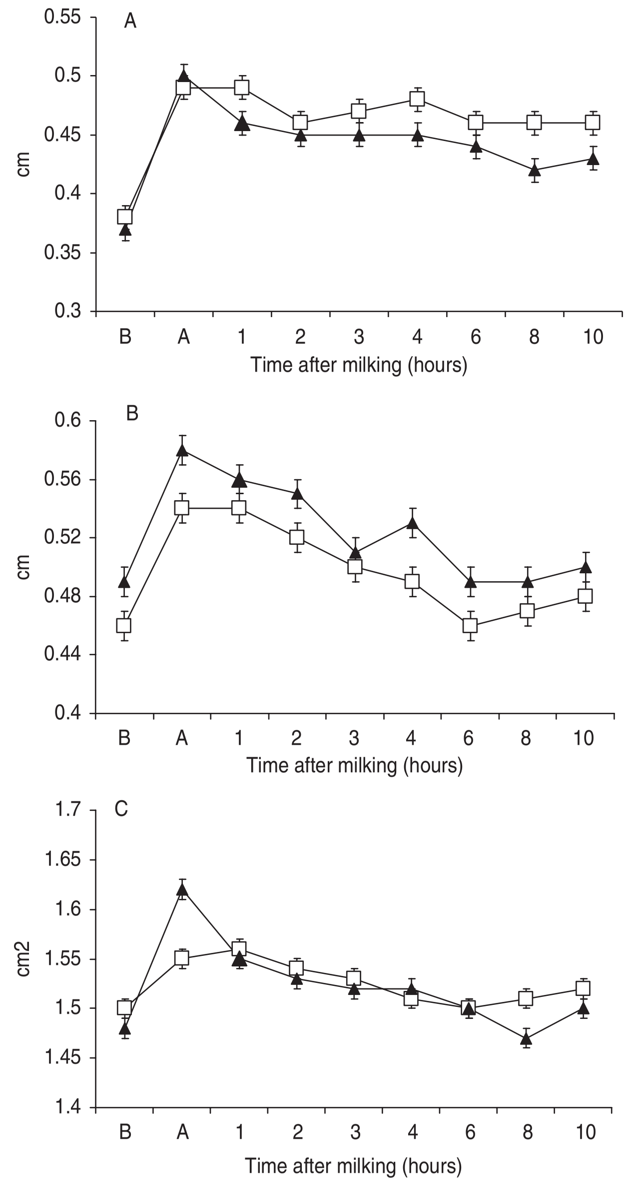

Fig. 1. Recovery time of teat wall thickness (A: Experiment 1, □ kid sucking; ▲ machine milking; B: Experiment 2, □ removal by catheter; ▲ machine milking) and teat wall area (C: Experiment 3, □ hand milking; ▲ machine milking) depends on milk removal method (before milking: B; after milking: A).

MM caused a significant increase in TWT compared with RC, whereas the remaining variables (TWA, TEWA and TCL) were not affected by the extraction method. In goats, Alejandro et al. (2014a) report that mechanically milked glands have higher TWT and TWA values compared with milk extraction by catheter, while Gleeson et al. (2002), in the bovine, note that milk extraction by catheter led to lower TWT and TCL values than machine milking.

In milk extraction by catheter, the only milk obtained is in the udder cistern and no compression force is exerted on the udder, so it was to be expected that the variables measured would not change after milking. Nevertheless, we observed

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that TWT increased compared with the pre-milking values, as was observed in bovine cattle (Gleeson et al. 2002) and goats (Alejandro et al. 2014a), so it seems reasonable to suppose that there are additional factors, other than milk extraction, which affect in teat tissue variation. When milk is extracted from the cistern by catheter, the intramammary pressure is decreased (Hamann & Mein, 1990), lowering the pressure on the mammary gland wall muscles (Lefcourt, 1982) and reducing stretching of the teat cistern walls, leading to an increase in TWT values. However, Hamann & Mein (1990) in bovine observed that RC caused a reduction of 3% in teat thickness, gauged by cutimeter. According to these authors, intramammary pressure does not affect measurements taken by cutimeter because the spring pressure of the device is higher than the internal blood pressure. On the other hand, with ultrasound no pressure is applied to the teat, so intramammary pressure could affect the TWT measurements (Alejandro et al. 2014a). These authors found an increase in TWT, TWA and TEWA following RC extraction in goats.

MM increased the TWT, TWA, TEWA and TCL values compared with hand milking. In goats (Alejandro et al. 2014a) and cows (Gleeson et al. 2002), hand milking leads to lower TWT values, possibly due to the different pressures applied to the teat or due to the manual teat stimulation during hand milking, may have activated some local regulatory mechanism in the udder (Svennersten et al. 1990). The force applied by the operator to raise the pressure and force intracisternal milk flow through the teat canal and extract the milk manually depends on the teat canal patency (McDonald & Witzel, 1966), and in this sense the vacuum needed to open the teat canal in Manchega ewes is much lower (17 kPa) than in goats (35 kPa, Díaz et al. unpublished data). This low pressure applied in the teat walls might explain the non-increase of some of the thickness variables measured after hand milking (TEWA and TCL) and the swift recovery of those that underwent a slight increase (TWT and TWA).

Teat recovery time

In high-production dairy sheep flocks, milking is usually applied twice daily (Castillo et al. 2008). After conventional milking, the teat needs a few hours to recover its pre-milking state, and during this period the risk of microorganisms penetrating the teat canal and, in consequence, the risk of new intramammary infections, is increased (Hamann & Osteras, 1994). In practical situations, evaluating the changes in teat tissues is the most suitable method to estimate the degree of bacterial penetrability in the teat (Neijenhuis et al. 2001). To this end, it is important to know the teat tissue recovery time to establish an optimum interval between milkings.

The highest TWT, TWA, TEWA and TCL values were obtained immediately after milk extraction and diminished as of 1 or 2 h after extraction, depending on the method (LS, RC, HM and MM). In goats, Alejandro et al. (2014a) and Fahr et al. (2001) also found that the greatest values were obtained after milking. In cattle, Neijenhuis et al. (2001) observed that the highest TWT value was obtained after milking and at 2 h in the case of TCL, whereas Ślósarz et al. (2010) and Wójtowski et al. (2006) obtained the highest TWT and TCL at 4 h after machine milking.

Teat recovery time varied depending on the extraction method, and the same method (machine milking) varied between the different trials. In EXP2 and EXP3 the TWT values were recovered between 4 and 6 h after milking. These values are similar to those reported by Wójtowski et al. (2006) in sheep, who reported that the TWT was recovered from 4 to 10 h after milking. In cattle, the TWT varied between 5 h (Gleeson et al. 2002) and 6 h (Neijenhuis et al. 2001). TWA presented a similar evolution to TWT, although the area of the walls was recovered 2 h (EXP2) and 1 h (EXP3) sooner than with mechanical milking.

TWT and TEWA were not recovered within the first 10 h after MM or LS (EXP1). This agrees with that found in goats by Alejandro et al. (2014a) who report that TWT and TWA are not recovered before 10 h with any extraction method (hand milking, mechanical milking, kid suckling and milk removal by catheter). The fact that TWT recovered at 4 and 6 h after MM in EXP3 and EXP2, respectively, but did not do so in EXP1 until after 10 h, even though the glands were milked in the same machine milking conditions, raises the hypothesis that the reason may be associated with the physiological features of the teats of the animals included in the experiment (closer to partum, offspring suckling on collateral gland, among others). This might also explain the non-recovery of TWT and TWA for more than 10 h in the teats suckled by lambs.

In the case of TEWA and TCL, the recovery time varied between the experiments. In EXP3 the values were recovered immediately after HM, whereas recovery time lasted up to 3 h with LS (EXP1) and 4 h with RC (EXP2). Wójtowski et al. (2006) indicate that TCL is recovered between 4 and 10 h after milking in sheep, although Alejandro et al. (2014a) in goats, affirm that TCL is not recovered before 10 h. In cattle, Gleeson et al. (2002) and Neijenhuis et al. (2001), report that TCL is recovered between 5 and 8 h after milking. According to Hamann & Mein (1990) and Gleeson et al. (2002), the differences in recovery time depend on the type of milking applied. However, the recovery time varied among trials using the milking method (machine milking), indicating that there are other factors that affect recovery time. Alejandro et al. (2014a) observed that the differences found in the teat tissue recovery pattern among the different physiological methods of extraction may be due to the differences in pressure exerted on the teat.

Conclusions

The increase in teat wall thickness and the recovery time for all the variables following mechanical milking was similar to that observed in comparison with the other three physiological methods of reference studied (lamb suckling, milk extraction by catheter and hand milking). So, it may be concluded that machine milking, carried out in optimum conditions and respecting the time interval between milkings usually applied on sheep farms (8–12 h), would not affect teat integrity. Moreover, given the variability observed in teat thickness recovery time between the different experiments, further research should be carried out to study which factors intrinsic and extrinsic to the animal may affect the teat wall thickness and recovery time after machine milking.

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References

Alejandro M, Roca A, Romero G & Díaz JR 2014a Effects of milk removal on teat tissue and recovery in Murciano-Grandina goats. *Journal of Dairy Science* (in press)

Alejandro M, Rodríguez M, Peris C & Díaz JR 2014b Study of ultrasound scanning as method to estimate changes in teat thickness due to machine milking in Manchega ewes. *Small Ruminant Research* **119** 138–145

Castillo V, Such X, Caja G, Casals R, Albanell E & Salama AA 2008 Effect of milking interval on milk secretion and mammary tight junction permeability in dairy ewes. *Journal of Dairy Science* **91** 2610–2619

Fahr R, Schulz J & Rosner F 2001 Melkbedingte Veränderungen an der Zitzenspitze der Ziege [Milking associated changes of the teat end in goats]. *Tierarztliche Praxis* **29** G151–G156

Gleeson DE, O'callaghan EJ & Rath MV 2002 Effect of milking on bovine teat tissue as measured by ultrasonography. *Irish Veterinary Journal* **55** 628–632

Hamann J & Burvenich C 1994 Physiological status of the bovine teat. En: Teat Tissue reactions to machine milking and new infection risk. *International Dairy Federation* **297** 3–11

Hamann J & Mein GA 1988 Responses of bovine teat to machine milking. Measurement of changes in thickness of the teat apex. *Journal of Dairy Research* **55** 331–338

Hamann J & Mein GA 1990 Measurement of machine-induced changes in thickness of the bovine teat. *Journal of Dairy Research* **57** 495–505

Hamann J & Osteras O 1994 II Special aspects. In Teat Tissue reactions to machine milking and new infection risk. *International Dairy Federation* **297** 35–41

Knizkova I, Kunc P, Broucek J & Kisac P 2005 The effect of calf suckling and machine milking on bovine teats. In *Physiological and Technical Aspects of Machine Milking Proceedings of the International Conference held in Nitra, Slovak Republic, 26–28 April*, pp. 137–146 (Eds V Tancin, S Mihina & M Uhrincat). Villa del Ragno, Via Nomentana 134, 00162 Rome, Italy: ICAR

Lefcourt AM 1982 Rhythmic contractions of the teat sphincter in bovines: an expulsion mechanism. *The American Journal of Physiology* **242** R181–R184

McDonald JS & Witzel DA 1966 Differential pressures across the bovine teat canal during three methods of milk removal. *Journal of Dairy Science* **49** 176–178

Neijenhuis F, Klungel GH & Hogeveen H 2001 Recovery of cow teats after milking as determined by ultrasonography scanning. *Journal of Dairy Science* **84** 2599–2606

Rasmussen MD & Mayntz M 1998 Pressuer in the teat cistern and the mouth of the calf during suckling. *Journal of Dairy Research* **65** 685–692

Ślósarz P, Wójtowski J, Bielińska S, Frąckowiak A, Ludwiczak A, Krzyżewski J, Bagnicka E & Strzałkowska N 2010 Machine induced changes of caprine teats diagnosed by ultrasonography. *African Journal of Biotechnology* **9** 8698–8703

Svennersten K, Claesson C & Nelson L 1990 Effect of local stimulation of one quarter on milk production and milk components. *Journal of Dairy Science* **73** 970–974

van der Tol PPJ, Schrader W & Aernouts B 2010 Pressure distribution at the teat-liner and teat-calf interfaces. *Journal of Dairy Science* **93** 45–52

Wójtowski J, Ślósarz P, Bielińska S, Nowicki S, Gut A & Danków R 2006 Ultrasound image of morphological changes of teat end in sheep caused by machine milking. *Archiv Tierzucht Dummerstorf* **49** (Special Issue) 231–237