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Assessing teat canal morphology in the dry period and during lactation by high-resolution ultrasound

Research Article

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Abstract

Our objectives were to quantify the dimensions of a fully 'closed' teat canal in dry cows and to describe recovery of the teat canal between milkings in lactating cows to assess whether and when full closure is attained, since this is an important determinant of udder health. Using an ultrasound scanner, teat canal length and diameter (proximal, midpoint and distal), teat cistern width, teat end width, whole teat width and teat wall thickness in 77 dry and 39 lactating dairy cows were measured. The dry cows represented a cross section of the dry population, with days since dry off ranging from 0 to 69 (median: 27). Data from lactating cows were recorded just before milking, and every 3 h post-milking. To control for location a cross-over (parlour *vs.* barn) study design was used. In dry cows, teat canal length and diameter did not vary by quarter or days since dry off, but multiparous cows had significantly wider teat canals than primiparous cows. The dry cow measurements can be used as baseline for dimensions for closed teats. In lactating cows, all teat dimensions except teat end width changed significantly during the 12-h milking cycle. Location (parlour *vs.* barn) did not affect the measurements, except teat end width and teat wall thickness. Teat canal length increased after milking and returned to pre-milking values by 9 h. Proximal and midpoint teat canal diameters decreased slightly just after milking and then progressively increased to above the pre-milking values by 9 h. Distal teat canal diameter increased after milking, partially contracting by 9 h. We found that during the dry period the teat canal is in a steady state, but its diameter is not zero, while during the lactation, the teat canal is in a near constant state of remodelling.

Mastitis, defined as inflammation of the mammary gland, compromises animal welfare and causes reduced milk yield (Halasa *et al.*, 2007). The teat end is the first line of defense against intramammary infection (IMI) and is important in preventing bacteria from invading the teat cistern (O'Shea, 1987). The teat canal is highly specialized in its anatomical structure, physiological functions and immunological defense mechanisms to prevent leakage of milk and entry of microorganisms. It is kept closed between milkings or suckling rounds by a smooth muscle sphincter that allows opening and closure of the distal teat canal orifice (Paulrud, 2005; Mein, 2012; Krömker, 2014). The teat canal is lined with stratified squamous epithelium, in which the superficial strata undergo keratinization and sloughing, resulting in constant regeneration of the inner teat canal lining transporting cellular detritus out of the teat. The epithelia are derived from ectoderm, the primary germ cell layer, and their function includes sealing of the teat canal during the dry period and between milkings, and physically blocking penetration of mastitis-causing microorganisms (Gruet *et al.*, 2001; Paulrud, 2005; Martin *et al.*, 2018).

Ultrasonography has been used to investigate bovine teat canal morphology and closure in lactating cows, with publications covering methodology (Neijenhuis *et al.*, 2001a, 2001b; Martin *et al.*, 2018; Wieland *et al.*, 2018), the impact of milking on the teat canal (Strapak *et al.*, 2017; Martin *et al.*, 2018) and the relationship between teat anatomy and milk flow (Weiss *et al.*, 2004). To date, only one study describes the use of ultrasound to assess teat morphology in dry cows (Toth *et al.*, 2019). It followed cows throughout the dry period to assess the regeneration of the 'streak canal' (that is, the teat canal length), and remodelling and recovery of the teat end in individual cows. Both the teat canal length and teat end area decreased in the first week of the dry period, with a further decrease in teat canal length in the last month of gestation (Toth *et al.*, 2019).

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Recovery of the teat canal between milkings has been examined previously. Significant changes in all teat traits except for teat end width were associated with the milking process in cows milked twice daily, including teat canal diameter and teat canal length (Martin *et al.*, 2018). After milking, teat canal diameter almost fully contracted to pre-milking values by 120 min, but this was not enough time for complete recovery of teat canal length (Strapak *et al.*, 2017). Teat end shape may affect time to recovery, which is faster for flat teats than for pointed teats, and may not be complete before the next milking for pointed teats, implying that they may sustain cumulative damage over time (Melvin *et al.*, 2019). However, none of these previous studies defined complete closure of the teat canal in lactating cows. Our hypothesis is that the ultrasound measurement of a dry cow teat canal can be used as baseline for teat end closure and hence for evaluating the degree of closure in lactating cow teat canals.

The aims of this study were to evaluate whether ultrasonography of dry cow teats would give insight into the closure of a teat canal, as during the dry period the canal should be quiescent. In addition, the dry cow data would help to understand whether ultrasound can be used to quantify teat canal closure between milking in lactating cows.

Materials and methods

Regulatory compliance

This research was approved by the Ethics and Welfare Committee of the School of Biodiversity, One Health and Veterinary Medicine of the University of Glasgow, Glasgow, UK (Ref EA25/19).

Dry cow study

Dry cow measurements were taken in July 2019 at an 850-cow commercial dairy farm in Scotland, UK. Whilst in lactation, cows were milked three times a day at 8-h intervals and fed a grass silage-based total mixed ration *ad libitum* with *ad libitum* access to water. The herd was housed all-year round and had an average whole herd production per cow per day of 39.5 l and 305d milk yield per cow of 12 100 l between August 2018 and July 2019. Predicted calving date was calculated using the herd management software with an assumed gestation length of 280 d. Average dry period length in the year preceding the study was 46 d (standard deviation: 14 d). Once a week, cows 42–49 d prior to predicted calving date were dried off. This was done in the parlour after the morning milking, with cows abruptly dried off and moved away from the main farm to a dry cow sand cubicle shed and fed a grass silage-based dry cow diet. The farm used a selective dry cow treatment regime. All cows received teat sealant in all four quarters (Cepralock®, MSD Animal Health, Bismuth Subnitrate, Milton Keynes, UK), but only cows with a SCC over 200 000 in at least one of the last three milk recordings, history of clinical mastitis in the current lactation or milk yield over 22 l at dry-off received intramammary antimicrobial treatment with a Cloxacillin-based product (Noroclox DC®, Norbrook, Cloxacillin Benzathine, Newry, UK). Between August 2018 and July 2019, 63% (366 of 581) of cows did not receive antimicrobial treatment at dry-off. Three weeks prior to calving, cows were moved to straw bedded calving pens.

A portable ultrasound machine (Mindray DP-30 VetTM, IMV imaging, Bellshill, UK) with a 10 MHz linear probe was used to scan either right hind and left front quarter or left hind and right front quarter, by the same operator throughout the study. Previous pilot studies showed that teat canal ultrasound measurements taken by a single observer are accurate and repeatable (results unpublished). A water bath, changed between teats, was used to prevent deformation of the teat by pressure from the probe. The teats scanned were selected based on convenience, including cow temperament and operator safety. However, an equal number of right hind/left front and left hind/right front images were obtained, to account for lying habits during late gestation as found in a previous behavioural study (Forsberg *et al.*, 2008).

In addition, external teat length measured with a ruler, teat shape and teat score were recorded. Teat shape was recorded as round, pointed or square (Wieland *et al.*, 2019). Teat end score was recorded on a scale of 1–4, correlating to the N, S, R, VR categorization, where 1 means no ring and a smooth teat end, 2 denotes presence of a smooth or slightly rough ring, 3 means a rough ring with keratin extending 1–3 mm from the orifice and 4 indicates a very rough ring with keratin extending 4 mm or more (Hillerton, 2005). Cow level data were collected from the milk recording Cattle Information Service and Dairy Comp 305, using data from the most recent lactation. These included lactation number (parity), dry-off date, expected calving date, days in milk (DIM) at dry-off, last recorded milk yield, fat percentage, protein percentage and somatic cell count (SCC). Additionally, projected 305-day milk yield was recorded.

Lactating cow study

The lactating cow study was conducted on four consecutive days in July 2019 at the University of Glasgow Research Farm, Scotland, U.K., with 19 or 20 cows scanned per day (details below). The dairy farm had 46 Holstein–Friesian cows housed in a 68 free stall cubicle shed. Cows were fed a partial mixed ration based on grass silage and in-parlour concentrate, with strip grazing of paddocks, weather permitting, from May to September. The average production per cow per day was 29 l, with a 305 d milk yield of 9500 l between August 2018 and July 2019.

Cows were milked as one group twice daily at 3.30 am and 3.30 pm in an 8/16 herringbone swing-over parlour (Alfalaval, Alfa Laval Ltd, Camberley, UK). Milking lasted around 1 h and 30 min. The system vacuum was set to 48 kPa, yielding an average claw vacuum during peak milk flow of 40 kPa. The pulsator (Alfalaval, Alfa Laval Ltd, Camberley, UK) was set up at a pulsation rate of 60 cycles/min with a pulsation ratio of 65:35. Clusters were removed automatically when milk flow decreased to below 0.4 kg/min. The milking liners, from Milkrite InterPuls (InterPuls UK, Melkham, UK), were made of rounded rubber, not ventilated, with an inner milk tube diameter of 10.5 mm. The long milk tube was silicone with a diameter of 15.5 mm and length of 3 m. The milking routine was performed by one operator per session. It consisted of pre-milking udder preparation for one side of the parlour (eight cows) with the following steps: dipping all four teats with iodine-based dip (Deosan Activate Barrier, Diversey, Fort Mill, South Carolina, USA), forestripping (three strips per teat) around 30 s after dipping. Once all eight cows had been prepared, the operator returned to the first cow, dried the four teats of each cow with a clean paper towel (one towel per cow) and attached the cluster. Following milking, cows were held for 30 min in a feeding area equipped with headlocks, then let into the bedded area to lie down for

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about 3 h before being let back out for strip grazing in the summer.

Ultrasound teat data were collected using the same machine and methodology as used for the dry cows, but in a cross-over study design with each cow scanned in two locations (barn/parlour): 19 cows were scanned in the barn, and 20 cows in the parlour on days 1 and 2, then the 20 cows were scanned in the barn and 19 in the parlour on days 3 and 4. In both locations, each cow was scanned at four timepoints; 5–10 min before milking (T0), 3 h post-milking, 6 h post-milking and 9 h post-milking. Unlike in the dry cows, in lactating cows the left front and right hind teat were used, as in previous studies (Neijenhuis *et al.*, 2001a; Wieland *et al.*, 2018, 2019).

Cow level data, except for dry-off date and expected calving date, were collected as described for dry cattle from the milk recording Cattle Information Service and Dairy Comp 305.

Data analysis

Stored images were analysed to collect data on teat canal length (TCL), teat canal diameter proximal (TCDP), teat canal diameter midpoint (TCDM), teat canal diameter distal (TCDD), teat cistern width (TCiW), teat end width (TEW), and teat wall thickness (TWT) (see online Supplementary Figs S1a and S1b for schematic representation and example, respectively). While the lactating cow images were analysed for all measurements, the dry cow images were examined only for TCL, TCDP, TCDM, TCDD due to interference of teat sealant with measurement of the teat cistern and a primary interest in teat canal closure. All measurements were recorded in centimetres as a function of the ultrasound software and converted to millimetres for ease of interpretation. In an exploratory analysis, we quantified linear relationships between the ultrasound measurements using Pearson correlation coefficients.

Linear mixed effects regression was used to investigate associations between the teat ultrasound measurements and covariates of interest. A cow identifier was included as random effect to account for dependence between multiple quarter measurements per cow, over time and between front/rear and left/right quarters measured on the same cow. Stepwise selection based on Akaike's information criterion was used to select variables for inclusion in the final regression models. Study design variables, and other covariates of primary interest, were forced into the model regardless of their effects on model fit. For the dry cows, the forced variables were quarter (left/right and front/rear), and number of days since dry-off. For the lactating cows, the forced variables were time in relation to milking, quarter, location (barn or parlour) and order of location (day 1 and 2 *vs.* day 3 and 4) to account for any familiarization effect. The nonlinear effects of DIM in lactating cows were modelled with cubic splines, using five knots (degrees of freedom) on the data interval. Splines are piecewise linear models that are more flexible than polynomial regression (Hastie *et al.*, 2009). Projected 305d milk yield was also included as a spline in each of the lactating cow regression models. Outlier observations were identified in the initial stages of model building and removed if they were not representative of the study population before fitting the final models. Bootstrap percentile confidence intervals, using 10 000 bootstrap replications, were calculated for the fitted regression model effects of time in relation to milking.

All analysis was performed in R (R Core Team, 2023). The linear mixed effect regression models were fitted with the *lme4* (Bates *et al.*, 2015) and *lmerTest* (Kuznetsova *et al.*, 2017) packages. We used the *DHARMa* package (Hartig, 2020) for residual analysis and other diagnostics checks of the final regression models. Results were considered statistically significant when *P*-values were less than 0.05.

Results

Dry cow results

Teat ultrasound measurements were made on 77 dry cows. Two cows, with 84 and 368 d since dry off, respectively, were considered outliers and removed from the analysis. Days since dry-off ranged from 0 to 69 d (median: 27) for the scanned cows. Thirty-four cows (45%) were in the dry period following first lactation, 22 cows (29%) following second lactation, and 19 cows (25%) had had three to five lactations, reflecting the composition of the herd. Per cow, two teats were scored, measured and scanned, giving teat measurements on $n = 150$ images for analysis. External teat length ranged from 2.1 to 6 cm (median: 4 cm). Most quarters (85%) were given teat scores of 1; teat scores of 2 (10%), 3 (1.3%) and 4 (3.3%) made up the remainder. Teat canal length ranged from 5.4 to 14.8 mm (median 9.2). The median proximal, midpoint and distal teat canal diameters were 3.0 mm (range: 1.6–5.1), 2.9 mm (range: 1.1–4.5) and 2.4 mm (range: 1.2–4.0), respectively. Descriptive statistics of the ultrasound measurements on teats from dry cows are summarized in Table 1 and Fig. S2.

There were moderately positive correlations between each of the teat canal diameters (TCDP, TCDM, TCDD), ranging from 0.33 between TCDP and TCDD, to 0.49 between TCDP and TCDM, but almost no correlation between teat canal diameters and teat canal length (Table S1, supplementary material). Quarter position (front/rear, left/right) and time since dry-off (Table 2; Fig. S3) were not significantly associated with any of the four teat canal measurements in the dry cows. TCDP was positively associated with shorter teat length ($P = 0.04$) and negatively associated with higher teat scores ($P = 0.01$). Cows with two or more lactations had significantly wider teat canals than first lactation animals.

Lactating cow results

Teat ultrasound measurements were made on 39 lactating cows. Two teats of each cow, left front (LF) and right rear (RR), were scanned four times in each of two locations (barn and milking parlour), giving a total of $n = 624$ ultrasound scans. Thirteen cows (33%) were primiparous and 26 (67%) were multiparous, between their second and eighth lactation. DIM at the ultrasound scan ranged from 8 to 557 d (median: 183). Projected 305d milk yield ranged from 6538 to 14 576 l (median: 10 261).

External teat length ranged from 2.8 to 6.4 cm (median: 4.5 cm). The median proximal, midpoint and distal teat canal diameters were 3.0 mm (range: 1.2–6.5), 2.8 mm (range: 0.9–6.5) and 2.5 mm (range: 0.9–6.1), respectively. Seventy-six of the measured teats (97%) were given teat scores of 1. The remaining two teats, from two different cows, were scored 2 and 4, respectively. Because all but two teats had teat score 1, this variable was not included in the regression models. Most teats had either a round (58%) or a square (41%) external shape. Only one teat with a pointed shape was observed. Descriptive statistics of the teat ultrasound measurements on the lactating cows are summarized in Table 1 and Fig. S2.

In the regression models for lactating cows, external teat shape was only associated with teat wall thickness (Table 3). However,

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Table 1. Descriptive statistics of the four teat ultrasound measurements (in mm; $n = 150$) made on 75 dry cows at 0 to 69 d post dry-off, the seven teat ultrasound measurements (in mm; $n = 624$) made on the lactating cows

	TCL	TCDP	TCDM	TCDD	TCiW	TEW	TWT
Dry cows							
Minimum	5.4	1.6	1.1	1.2			
1st Quartile	8.0	2.6	2.5	2.1			
Median	9.2	3.0	2.9	2.4			
3rd Quartile	10.8	3.5	3.2	2.8			
Maximum	14.8	5.1	4.5	4.0			
Mean	9.4	3.0	2.9	2.4			
Std deviation	1.9	0.6	0.6	0.6			
Lactating cows							
Minimum	5.5	1.2	0.9	0.9	3.1	16.9	3.8
1st Quartile	9.6	2.5	2.4	2.0	8.8	21.1	6.6
Median	10.9	3.0	2.8	2.5	11.4	22.9	7.5
3rd Quartile	12.3	3.4	3.2	2.9	13.6	24.6	8.5
Maximum	20.4	6.5	6.5	6.1	26.8	29.4	12.9
Mean	11.1	3.0	2.8	2.5	11.3	23.0	7.6
Std deviation	2.1	0.7	0.7	0.7	3.4	2.4	1.4

TCL, Teat canal length; TCDP, Proximal teat canal diameter; TCDM, Midpoint teat canal diameter; TCDD, Distal teat canal diameter; TCiW, Teat cistern width; TEW, Teat end width; TWT, Teat wall thickness.

Table 2. Regression coefficients and *P*-values for variables included in the final linear mixed effects regression models for dry cows

	TCL	TCDP	TCDM	TCDD
Left vs. Right quarter (baseline = Left)	+0.25 $P = 0.35$	+0.07 $P = 0.46$	+0.13 $P = 0.20$	+0.08 $P = 0.38$
Front vs. Rear quarter (baseline = Front)	−0.23 $P = 0.39$	−0.007 $P = 0.95$	−0.066 $P = 0.52$	+0.064 $P = 0.47$
Days since dry-off	+0.004 $P = 0.66$	−0.003 $P = 0.18$	−0.002 $P = 0.33$	−0.0003 $P = 0.87$
External teat length (cm)	–	0.15 $P = 0.04$	–	–
Teat score (1 vs. 2 +) (baseline = 1)	+0.03 $P = 0.95$	−0.37 $P = 0.01$	N/A[a]	−0.03 $P = 0.83$
Lactation group (baseline = 1)	+0.31 $P = 0.37$	+0.26 $P = 0.02$	N/A[a]	+0.30 $P < 0.01$

Variables were forced into the model (left/right, front/rear, days since dry off) or included based on statistical significance. Dashes (–) indicate that the covariate was not included in the final model. TCL, Teat canal length (mm); TCDP, Proximal teat canal diameter (mm); TCDM, Midpoint teat canal diameter (mm); TCDD, Distal teat canal diameter (mm).
[a]Not available. Due to the particular sample values for TCDM, inclusion of Lactation Group and/or Teat Score causes model singularities.

the single pointed teat significantly influenced the regression coefficients for the teat shape categories (pointed, round, and square), and we therefore decided to exclude the pointed teat data from the TWT model.

In the lactating cows, correlations between the teat canal diameters ranged from 0.30 to 0.49 (Table S2, supplementary material). Teat canal length and teat end width were positively correlated ($r = 0.43$), reflecting overall teat end size. Teat cistern width and teat wall thickness were negatively correlated ($r = -0.53$). Statistically significant changes were observed in teat canal length, diameter, cistern width, and teat wall thickness (Table 3), in relation to milking time. Compared to the scan immediately prior to milking (T0), teat canal length was increased at 3 and 6 h after milking, with almost full contraction to the T0 teat length by 9 h post-milking (Fig. 1). Proximal teat canal diameter decreased through 3–6 h after milking, but fully dilated by 9 h. Distal teat canal diameter showed the opposite trend, increasing after milking, and partially contracting by 9 h. The midpoint teat canal diameter increased at 9 h beyond the milking time (T0) measurement. Teat cistern width showed a significant decrease at 3 and 6 h after milking, but almost fully returned to T0 width by 9 h post-milking. Teat wall thickness increased after milking, also returning to pre-milking values by 9 h post-milking. Teat end width did not show significant change in the time points following milking.

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Table 3. Regression coefficients and *P*-values for variables included in the final linear mixed effects regression models of the seven teat ultrasound measurements made on the lactating cows

	TCL	TCDP	TCDM	TCDD	TCiW	TEW	TWT
Time (polynomial)	$P<0.01$	$P<0.01$	$P<0.01$	$P=0.01$	$P<0.01$	$P=0.69$	$P<0.01$
Quarter (baseline = left front teat)	+0.32 $P=0.06$	−0.10 $P=0.06$	−0.12 $P=0.02$	−0.18 $P<0.01$	−0.27 $P=0.28$	−1.00 $P<0.01$	−0.46 $P<0.01$
Location (baseline = Barn)	−0.01 $P=0.94$	−0.06 $P=0.27$	−0.04 $P=0.47$	−0.09 $P=0.06$	−0.03 $P=0.86$	−0.23 $P=0.02$	−0.28 $P<0.01$
Turn (baseline = 1st turn)	+0.15 $P=0.21$	−0.10 $P=0.06$	−0.11 $P=0.03$	−0.10 $P=0.05$	+0.23 $P=0.20$	+0.19 $P=0.04$	−0.01 $P=0.91$
External teat length	+0.37 $P=0.03$	–	–	–	+0.99 $P<0.01$	–	–
External shape (baseline = Round)	–	–	–	–	–	–	+0.40 $P=0.04$
Lactation group (baseline = 1)	+1.1 $P=0.07$	−0.06 $P=0.62$	+0.06 $P=0.53$	+0.16 $P=0.20$	−0.68 $P=0.44$	+2.09 $P=0.01$	+0.92 $P<0.01$
DIM at US scan (spline)	$P<0.01$	$P=0.10$	$P=0.27$	$P=0.71$	$P<0.01$	$P=0.49$	$P<0.01$
Projected 305 milk yield (spline)	$P=0.08$	$P=0.63$	$P=0.12$	$P=0.28$	$P=0.01$	$P=0.63$	$P=0.21$

Dashes (–) indicate that the covariate was not included in the final model. TCL, Teat canal length (mm); TCDP, Proximal teat canal diameter (mm); TCDM, Midpoint teat canal diameter (mm); TCDD, Distal teat canal diameter (mm); TCiW, Teat cistern width (mm); TEW, Teat end width (mm); TWT, Teat wall thickness (mm).

Right rear teat canals tended to be longer than those in left front teats ($P=0.06$). All three teat canal diameter measurements, teat end width and teat wall thickness of the former were smaller than the latter, but teat cistern width did not differ significantly between the two. There were some differences between the teat measurements conducted at the two locations. Teat end width ($P=0.02$) and teat wall thickness ($P<0.01$) were smaller in the parlour, compared to the barn and distal teat canal diameter was also numerically (non-significantly, $P=0.06$) smaller in the parlour. For all three teat canal diameters, measurements at the first scanning were larger than at the second scanning turn. Teat end width was also larger ($P=0.04$) on the second scanning relative to the first.

Of the other covariates examined, external teat shape was associated with teat wall thickness. Square-shaped teats had thicker teat walls than round teats ($P=0.04$). Longer teats had longer teat canals ($P=0.03$) and larger cistern widths ($P<0.01$) than shorter teats. More DIM was associated with longer teat canals ($P<0.01$), thicker teat walls ($P<0.01$), and decreased teat cistern width ($P<0.01$). Projected 305d milk yield was associated with teat cistern width ($P=0.01$). First lactation cows had narrower teat ends ($P=0.01$) and thinner teat walls ($P<0.01$), compared to cows with more lactations.

Discussion

Here we demonstrate that ultrasound measurement of closed teats canals is feasible in dry cows. This was achieved by obtaining measurements of teat canal length, and three diameters along the canal as indicators of closure. Closure is not seen on ultrasound as a teat diameter of zero, rather, dry cows reach a minimum teat diameter which is consistent between front and rear or left and right quarters of the udder.

Although ultrasound has previously been used to examine the mammary cistern in dry cows (Bonelli *et al.*, 2020), only one other study has used ultrasound to examine the teat canal in dry cows. The results of that longitudinal study (Toth *et al.*, 2019) differ from ours in that while they saw a decrease in teat canal length we found no significant changes to the teat canal dimensions, either teat canal length or diameters, during the dry period (0–69 d). The lack of a change in our study could reflect the fact that we utilized a cross-sectional study design and therefore variation between cows might have masked any difference across the dry period.

Ranges of teat canal length and diameters of dry cows and lactating cows in our study overlap, but no direct comparison was made because the cows were managed in two different farming systems. In lactating cows, teat canal dimensions changed significantly over the milking cycle, as expected. A steady recovery of the teat canal length up to the final measurement at 9 h was observed but it did not reach pre-milking values, as previously observed in herds with 3× daily milkings where teat canal length took more than 8 h to recover (Neijenhuis *et al.*, 2001a; Melvin *et al.*, 2019; Wieland *et al.*, 2019). Repetition of this phenomenon at consecutive milking cycles would imply that teat canals continue to increase in length. Alternatively, if T0 is also treated as 12 h (ie the time of the next milking) the final 3 h in the milking interval would allow for the return to pre-milking length.

Proximal teat canal diameter fully recovered by 9 h, while the distal teat canal diameter only partially recovered by 9 h. However, the midpoint teat canal diameter was generally larger at 9 h than at all earlier time points measured in the milking interval. Previous research in 3× milking herds found that teat canal diameter at the proximal and middle regions increased near the end of the 8-h milking interval, but the distal measurement did not change (Melvin *et al.*, 2019). According to other studies, 2 h was enough time for the diameter to return to pre-milking values (Strapak *et al.*, 2017). In our lactating cow regression model, the fitted curve for midpoint teat canal diameter (Fig. 1) leads to a biologically implausible conclusion, namely that that teat canal midpoint keeps stretching wider between 9 h and the start of the next milking interval. This apparent effect is caused, firstly, by modelling TCDM as a second-order polynomial, and secondly, by the lack of higher time-resolution

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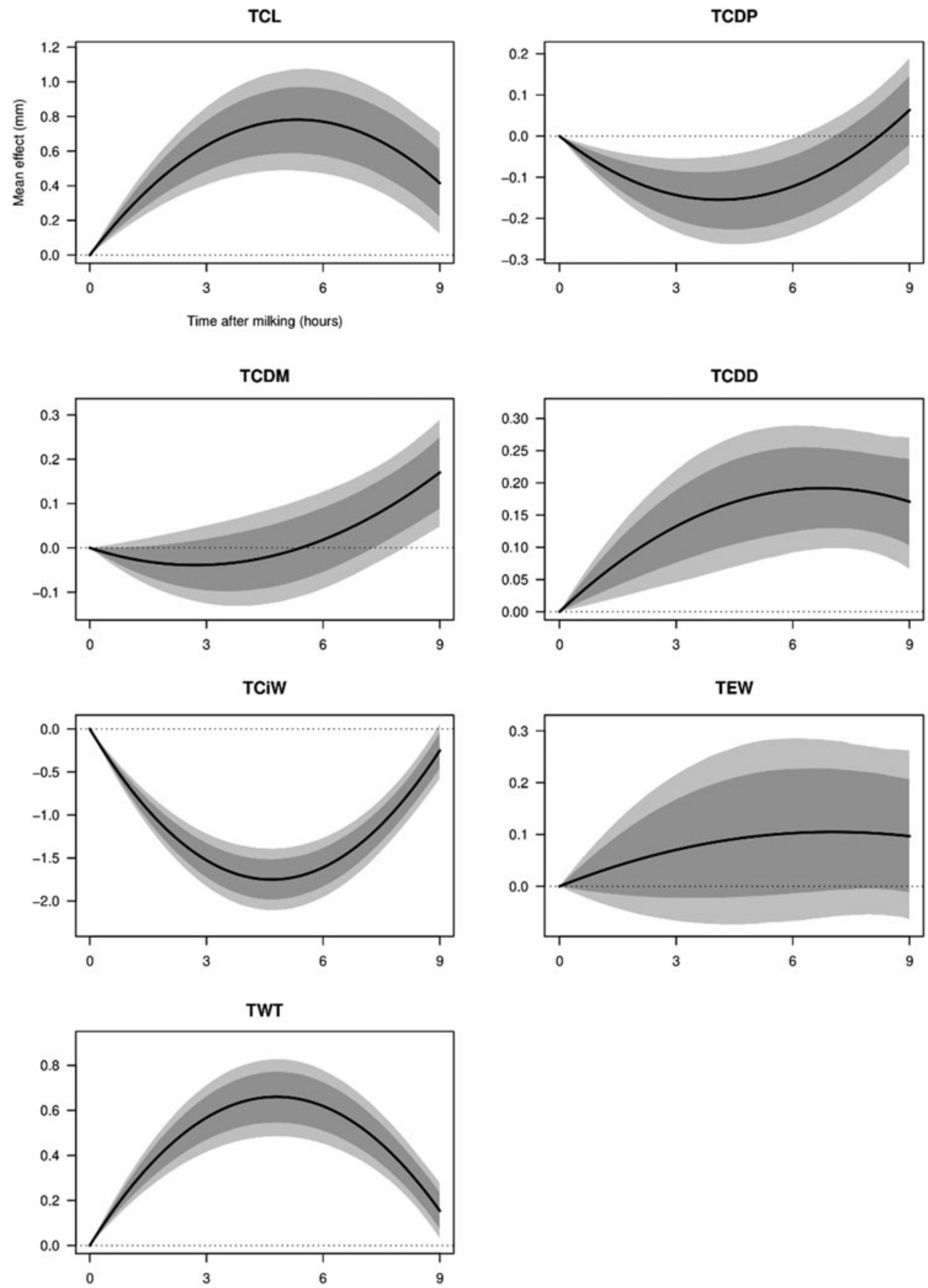

Figure 1. Mean modelled time effects (in mm) for the teat ultrasound measurements in lactating cows, starting from time T0 (just before milking) as baseline. Shaded regions show 95% bootstrap confidence intervals (10 000 replications) for the means; darker shaded regions are 80% confidence intervals. TCL, Teat canal length; TCDP, Proximal teat canal diameter; TCDM, Midpoint teat canal diameter; TCDD, Distal teat canal diameter; TCiW, Teat cistern width; TEW, Teat end width; TWT, Teat wall thickness.

data between 6 and 12 h post-milking. Future studies can address these issues by taking hourly TCDM measurements in the 6–12 h post-milking, and using a more flexible functional form, such as a spline or higher-order polynomial, for TCDM in the regression model.

In our study, teat end width did not change significantly in the interval between milkings. A small but significant increase in teat end width was found in a previous study, from pre-milking values of 21.2 mm to a maximum post-milking value of 22 mm, with return to pre-milking values taking longer than 8 h (Neijenhuis *et al*., 2001a).

As anticipated, TCiW in lactating cows changed over time, decreasing at 3 and 6 h after milking and fully increasing to pre-milking values by 9 h post-milking, and had a negative correlation with TWT ($r = -0.53$, Table S2). Recovery time of TWT and TCiW has previously been reported to be 6 and 8 h, respectively (Neijenhuis *et al*., 2001a), although some suggest they may return to pre-milking values as quickly as 3 h in the rear teats (Wieland *et al*., 2019). The highest level of change in teat cistern width occurred 3–6 h after milking, in accordance with data from other ultrasound-based evaluations (Martin *et al*., 2018). This is likely due to tissue elasticity and milk volume changes; after milk leaves the teat cistern, the teat wall thickens as the cistern volume decreases. Teat cistern dimensions are associated with teat position, with wider cisterns in front teats than rear teats (Neijenhuis *et al*., 2001a). While we found the same relation in lactating cows between quarter position and TCiW, it was not statistically significant.

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In contrast to the dry cows in our study, the lactating cows had significantly smaller teat canal measurements in the rear than in the front teats. Lactating cow protocols for teat canal ultrasound routinely focus on left front and right hind teats (Neijenhuis, Klungel, and Hogeveen, 2001a; Wieland *et al*., 2018; Wieland *et al*., 2019), and we assumed the observed association to be due to front *vs.* hind position rather than left *vs.* right position. Considering that we observed a left–right difference in dry cows, future lactating cow studies should probably consider both diagonally opposed quarter pairs. There is also the possibility that milking time and teat measurements are influenced by parlour type, milking cluster adjustment, or improper alignment of the milking machine with the teats during milking, often resulting from the weight of hoses and inadequate support.

Because cows are conditioned to respond to the milking parlour, which may affect pressure in the udder and teat shape, we compared measurements from images collected inside the parlour to those collected outside the parlour. We found all parlour measurements on average smaller than the barn ones, with TEW and TWT significantly smaller. We could speculate that reduced TWT is due to milk let-down, which stretches the teat but that would not explain smaller measurements for the teat canal diameters. TEW was slightly increased on the second scanning turn, whereas teat canal diameters were significantly narrower on the second turn, regardless of location or time. This was observed also at T0, when the cows were scanned just prior to milking, and the usual milking protocol (teat dipping, milk stripping, drying) had not been carried out, perhaps limiting the conditioned response. The reason for this is unclear, as we do not expect cows to so quickly dissociate teat handling for scanning from teat handling for milking. Given that the same ultrasound machine was used throughout, the observed differences between the scanning turns are most likely due to an observer effect; therefore, an artefact of our methodology.

Lactation groups differed with regards to TCL, TEW and TWT, with an increase in all values with two or more lactations. Dry cows with two or more lactations had significantly wider teat canals than first lactation animals. The first lactation takes place while the cow is still maturing and has narrower teat ends and thinner teat walls. By the second lactation, the cow is fully mature and the udder fully grown, with slightly larger teats. In general, parity is related to production level, which is also associated with TEW and TWT. In our study, this was visible in the data for DIM and 305 d yield: TEW showed an initial decrease until around 50–70 DIM, an increase until around 140 DIM, and stabilizes thereafter. TCiW followed a similar trend, with lowest values coinciding with the period of peak milk production for cows, usually around 50–70 DIM.

In conclusion, we demonstrated that teat canals of dry cows can be measured with ultrasound, and that these closed teats canals have non-zero diameters. Therefore, caution is needed when defining and interpreting degree of closure in lactating cow teat canals, if measured with ultrasound. While we found no significant changes in the dry cow teat canals over time, in lactating cows all teat dimensions measured, except for teat end width, changed significantly during each milking cycle. Further studies are required to refine the methodology, including evaluation of the potential association between cow or operator experience and measurements, scanning of both diagonally opposed teat pairs, and inclusion of additional time points or functional forms in modelling to explain the apparent non-return to baseline value for selected measurements.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029924000098

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