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**An observational study investigating the association of ultrasonographically assessed machine milking-induced changes in teat condition and teat-end shape in dairy cows**

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**Supplementary Material S1**

*Standard operating procedure for measurements of teat parameters*

Measurements were consistently performed in the same sequence according to the following protocol: Teat canal length (**TCL**) was defined as the distance between the visible proximal end at the aspect of the Furstenberg’s rosette and the distal end of the teat at the aspect of the teat orifice. Teat-end diameter (**TED**) was measured perpendicularly to the TCL at the proximal end of the teat canal and defined as the distance between both opposing exterior teat walls. The midpoint of the TCL was identified with an additional measurement to assess the teat-end diameter at the midpoint between the distal and the proximal end of the teat canal (**TMD**) which was measured perpendicularly to the TCL and defined as the distance between both opposing external teat walls. To assess teat cistern width (**TCW**), a line of 1 cm length was drawn alongside the longitudinal axis of the entire teat which differed in some but not all teat scans from the axis of the TCL. Teat cistern width was defined as the distance between both opposing internal teat walls 1 cm above the proximal end of the teat canal and measured perpendicularly to the teat axis. Teat wall thickness was assessed at the caudal teat wall 1 cm proximal to the Furstenberg’s rosette using the TCW as a landmark. Teat wall thickness was defined as the shortest distance between the external and the internal teat wall.

**Supplementary Material S2**

*Assessment of repeatability of triplicate measurements for teat length and teat diameter*

Repeatability of triplicate measurements for teat length and teat diameter, respectively, was evaluated by calculating concordance correlation coefficients (CCC) for nonlongitudinal repeated measurements as described by Carrasco *et al.* (2013) in R Statistical Software (R Core Team, 2015). Results of CCC were classified as poor (<0.40), fair (0.40 to 0.59), good (0.60 to 0.74), and excellent (0.75 to 1.00) according to Cicchetti (1994). Concordance correlation coefficients (95% confidence intervals) for triplicate measurements were 0.79 (0.76 to 0.82) for teat length and 0.43 (0.37 to 0.48) for teat diameter indicating excellent and fair agreement, respectively.

**Supplementary Material S3**

*Descriptive statistics and results of linear regression models for the relative change of teat-end diameter at the aspect of the proximal end of the teat canal, teat-end diameter at the midpoint between the distal and proximal ends of the teat canal, and the ratio of teat wall thickness divided by teat cistern width compared with the ratio before unit attachment*

*Teat-end diameter*. Average [mean ± SD (median; range)] TED was 21.9 ± 1.7 (22.0; 17.0 to 28.0) mm. The final multivariable model for the relative change of TED (**RCTED**) contained teat-end shape (*P* < 0.0001), time (*P* < 0.0001), the interaction term between teat-end shape and time (*P* = 0.0002), teat length (*P* = 0.04), teat diameter (*P* < 0.0001), quarter position (*P* < 0.0001), milking unit-on time (*P* = 0.001), and the premilking value at t-1 (*P* < 0.0001). A 1 mm increase in teat length increased the RCTED by 0.02 % (95% confidence interval (**CI**) 0.001 to 0.05). A 1 mm increase in teat diameter increased the RCTED by 0.3% (95% CI 0.2 to 0.4). Controlling for all other variables included in the model, the RCTED in front teats was 1.3% (95% CI -1.5 to -1.0) lower compared to hind teats. A 1 unit increase of the premilking value at t-1 decreased the RCTED by 1.2% (95% CI -1.2 to -1.1). The RCTED (LSM, 95% CI) at t0 was -2.5 (-3.5 to -1.6), -0.5 (-1.5 to 0.5), and -1.2 (-1.9 to -0.5) % in teats with pointed, flat, and round teat-end shapes, respectively, and was different between teats with pointed and flat (*P* = 0.0009) and pointed and round teat-end shape (*P* = 0.004). The recovery time for RCTED was 3 h in teats with pointed and 5 h in teats with round teat-end shapes, whereas RCTED was not different from the premilking value at a significance level of 0.05 in teats with flat teat-end shape (Supplementary Figure S1).

*Teat-end diameter at the midpoint between the distal and the proximal end of the teat canal.* Average [mean ± SD (median; range)] TMD was 18.8 ± 1.7 (19.0; 12.0 to 25.0) mm. The final multivariable model for the relative change of TMD (**RCTMD**) contained teat-end shape (*P* < 0.0001), time (*P* = 0.15), the interaction term between teat-end shape and time (*P* = 0.96), teat length (*P* < 0.0001), teat diameter (*P* = 0.0006), milking unit-on time (*P* < 0.0001), and the premilking value at t-1 (*P* < 0.0001). A 1 mm increase in teat length decreased the RCTMD by 0.1% (95% CI -0.1 to -0.04). A 1 mm increase in teat diameter increased the RCTMD by 0.1% (95% CI 0.1 to 0.2). For every 60 s increase in milking unit-on time, RCTMD increased by 0.4% (95 CI 0.3 to 0.6). A 1 unit increase of the premilking value at t-1 decreased the RCTMD by 1.6% (95% CI -1.7 to -1.5). The RCTMD (LSM, 95% CI) at t0 was 0.7 (-0.3 to 1.7), 4.1 (3.1 to 5.2), and 2.1 (1.5 to 2.7) % in teats with pointed, flat, and round teat-end shape, respectively, and was different between teats with pointed and flat (*P* = 0.0003), pointed and round (*P* = 0.02), and teats with flat and round teat-end shape (*P* = 0.0003). The recovery time for RCTMD exceeded the 7 h of observation in teats with flat and round teat-end shapes, whereas RCTMD was not different from the premilking value at a significance level of 0.05 in teats with pointed teat-end shape (Supplementary Figure S2).

*Relative change in ratio between teat wall thickness and teat cistern width*. The mean ± SD (median; range) TCW was 11.5 ± 3.4 (12.0; 1.0 to 23.0) mm. The final multivariable model for the relative change of the ratio of teat wall thickness divided by teat cistern width compared with the ratio before unit attachment (**RCTWCR**) contained teat-end shape (*P* < 0.0001), time (*P* < 0.0001), the interaction term between teat-end shape and time (*P* = 0.73), teat length (*P* < 0.0001), teat diameter (*P* < 0.0001), quarter position (*P* = 0.0001), and the premilking value at t-1 (*P* < 0.0001). A 1 mm increase in teat length decreased the RCTWCR by 0.9% (95% CI -1.4 to -0.5). A 1 mm increase in teat diameter decreased the RCTWCR by 2.3% (95% CI -3.5 to -1.2). Controlling for all other variables included in the model, the RCTWCR in front teats was 11.9% (95% CI 5.9 to 17.9) higher compared to hind teats. A 1 unit increase of the premilking value at t-1 decreased the RCTWCR by 10.2% (95% CI -31.5 to -25.5). The RCTWCR (LSM, 95% CI) at t0 was 69.1 (50.2 to 88.0), 56.8 (36.8 to 76.7), and 86.5 (72.7 to 100.3) % in teats with pointed, flat, and round teat-end shapes, respectively, and was different between teats with flat and round teat-end shapes (P = 0.002). Recovery time for RCTWCR was 3 h in teats with pointed, 1 h in teats with flat, and 3 h in teats with round teat-end shape, respectively (Supplementary Figure S3).

**Supplementary Table S1.** *Descriptive statistics of total milk yield (TMY, kg), average milk flow rate (AMF, kg/min), peak milk flow rate (PMF, kg/min), average milk flow rate between 60 and 120 s after start of milking (MF60-120, kg/min), and milking unit-on time (MUT, s) from four consecutive days in 125 cows stratified by milking session.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Milking 1 | | | | |  | Observational milking1 | | | | |  | Milking 3 | | | | |
| Item |  | Mean ± SD |  | Median |  | Range |  | Mean ± SD |  | Median |  | Range |  | Mean ± SD |  | Median |  | Range |
| TMY |  | 16.0 ± 4.7 |  | 16.6 |  | 4.2-40.1 |  | 8.0 ± 3.2 |  | 8.2 |  | 0.8-16.1 |  | 16.3 ± 4.8 |  | 16.3 |  | 0.4-35.9 |
| AMF |  | 3.6 ± 1.0 |  | 3.6 |  | 1.0-7.5 |  | 2.7 ± 1.1 |  | 2.8 |  | 0.4-6.0 |  | 3.6 ± 0.9 |  | 3.6 |  | 0.9-6.9 |
| PMF |  | 4.9 ± 1.2 |  | 4.8 |  | 1.0-7.5 |  | 3.9 ± 1.3 |  | 4.0 |  | 0.7-8.7 |  | 4.8 ± 1.2 |  | 4.8 |  | 0.3-10.1 |
| MF60-120 |  | 3.9 ± 1.3 |  | 4.0 |  | 0.4-8.2 |  | 2.7 ± 1.5 |  | 2.9 |  | 0.1-6.5 |  | 4.2 ± 1.1 |  | 4.2 |  | 0.2-9.6 |
| MUT |  | 272 ± 77 |  | 263 |  | 104-612 |  | 181 ± 51 |  | 173 |  | 86-373 |  | 278 ± 86 |  | 261 |  | 117-588 |

1Milking session when ultrasonographic scanning was performed.

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**Supplementary Figure S1** Relative change of the teat-end diameter of the left front and right hind teat in 125 cows measured immediately after milking unit detachment (t0) and 1, 3, 5, and 7 h after milking (t1, t3, t5, t7) compared with the measurement immediately before milking unit attachment (t-1). The results are stratified according to teat-end shape (pointed, flat, and round). Filled symbols indicate differences from 0 at the level of *P* < 0.05. Teat-end shape *P* < 0.0001, Time *P* < 0.0001, Teat-end shape × Time *P* = 0.0002. The results are presented as the least squares means (%) of repeated measures ANOVA and controlled for the effect of the teat length (*P* = 0.04), teat diameter (*P* < 0.0001), quarter position (*P* < 0.0001), milking unit-on time (*P* = 0.001), and premilking value at t-1 (*P* < 0.0001). Error bars show the 95% confidence intervals.

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**Supplementary Figure S2** Relative change of the teat-end diameter at the midpoint between the distal and the proximal end of the teat canal of the left front and right hind teat in 125 cows measured immediately after milking unit detachment (t0) and 1, 3, 5, and 7 h after milking (t1, t3, t5, t7) compared with the measurement immediately before milking unit attachment (t-1). The results are stratified according to teat-end shape (pointed, flat, and round). Filled symbols indicate differences from 0 at the level of *P* < 0.05. Teat-end shape *P* < 0.0001, Time *P* = 0.15, Teat-end shape × Time *P* = 0.96. Results presented as the least squares means (%) of repeated measures ANOVA and controlled for the effect of the teat length (*P* < 0.0001), teat diameter (*P* = 0.0006), milking unit-on time (*P* < 0.0001), and premilking value at t-1 (*P* < 0.0001). Error bars show the 95% confidence intervals.

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**Supplementary Figure S3** Relative change of the ratio of the teat wall thickness divided by teat cistern width of the left front and right hind teat in 125 cows measured immediately after milking unit detachment (t0) and 1, 3, 5, and 7 h after milking (t1, t3, t5, t7) compared with the measurement immediately before milking unit attachment (t-1). Results are stratified according to teat-end shape (pointed, flat, and round). Filled symbols indicate differences from 0 at a level of *P* < 0.05. Teat-end shape *P* < 0.0001, Time *P* < 0.0001, Teat-end shape × Time *P* = 0.73. Results presented as least squares means (%) of repeated measures ANOVA and controlled for the effect of teat length (*P* < 0.0001), teat diameter (*P* < 0.0001), quarter position (*P* = 0.0001), and the premilking value at t-1 (*P* < 0.0001). Error bars show the 95% confidence intervals.

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