- 1 Supplemental material to:
- 2

Effects of milking, over-milking and vacuum levels on front and rear quarter
teats in dairy cows

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7 Material and Methods

• Farms and animals

9 The study was conducted on 41 Austrian dairy farms. The farms were located in the Austrian federal 10 states Styria, Carinthia, and Burgenland. All farmers in the area which is covered by the veterinary 11 practice where two authors are working (TV and WP) were invited to participate. Since 12 measurements had to be taken during milking only farms with conventional milking systems were 13 included in the study, farms milking with automatic milking systems were not suitable for the study. 14 Thirty one milking parlours had low milk lines (399 cows) and 10 parlours (141 cows) were equipped 15 with high milk lines.

Overall 41 farms took part at the study. The herd size ranged from 16 to 107 cows per farm. The milking parlours were tandem (N = 11), herringbone (N = 19), swing over (N = 9) and side by side (N = 2) designs. An automatic pre-milking stimulation was installed on 22 farms. The clusters were automatically removed at 15 farms when the milk flow rate was lower than 0.2 kg/minute. Before any measurements were taken the farmers performed the routine cleaning of the teats (dry cleaning N = 18, moist cleaning N = 23) and additional fore-stripping was done on 17 farms.

The study comprised 540 dairy cows (61.9% Austrian Simmental, 23.5% Holstein Friesian, 14.6%
Brown Swiss). Cows with clinical mastitis, obvious udder malformation and injuries at the udder were

excluded from the study. The cows were between 55 and 256 days in milk and milked twice daily; the
daily milk yield ranged from 4.0 to 66.5 kg.

• Vacuum level measurement using VaDia vacuum logger

27 Two cluster, one on the right and one on the left side of the milking parlour were equipped with the 28 measurement vacuum device (supplement figure 1). The lightweight battery-operated portable 29 vacuum-logger (VaDia, BioControl, Rakkestad, Norway, 90 x 60 x 30 mm, 85 g) has four vacuum 30 measurement channels and records the vacuum levels 200 times per second per channel. All 31 obtained data during the milking time were stored automatically on the VaDia device. At the end of 32 milking the data was transferred to computer for further evaluation using the VaDia suite software 33 (BioControl, Rakkestad, Norway). The recorded data included duration of milking and over-milking, 34 vacuum level in the SMT and the vacuum levels in the front and rear MPC. The complete milking 35 process of each individual cow was divided into 4 milking phases (cluster attachment/stimulation, 36 milking/peak milk flow, over-milking, cluster removal/end of milking) which were automatically 37 detected by the VaDia device (VaDia User manual v3.7. www.biocontrol.no/vadia). The start of 38 milking was defined as the moment, when the vacuum in the SMT increased to 25 kPa. The milking 39 period is characterized by a stable vacuum. Over-milking starts when the vacuum variations in the 40 mouth piece chamber became equal or higher than 1.3 times the preceding running average 41 variation during milking period. The cluster take-off phase was characterized by a sudden decrease of 42 the vacuum in the MPC and a vacuum fluctuation in the SMT. The end of the milking was reached 43 when the vacuum in the SMT decreased to a level below 5 kPa.

• Statistical analysis

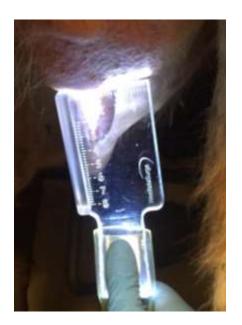
The statistical analyses were performed with SPSS software (Version 24.0, IBM SPSS Inc., Munich,
Germany) and with Excel 2017 (Microsoft Office Corporation, Redmond, WA).

• Ethical statement

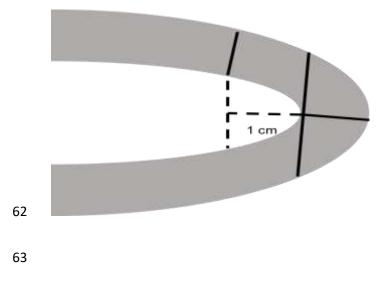
- 48 The study protocol has been approved by institutional and governmental animal welfare committees.
- 49 During the study no negative effects on the animals have been observed.
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- 51 Supplement figures



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- 53 Supplement figure 1: Milking cluster with installed VaDia for measurement of the vacuum
- 54 levels in the short milk tube, the mouth piece chambers and the pulsation tube



- 58 Supplement figure 2: Measurement tool with integrated light source and ruler for morphologic
- 59 measurements (teat length and teat width at the teat base)



Supplement figure 3: Ultrasonographic measurements before and after milking: teat canal
length, diameter of teat at internal teat canal orifice (Fürstenberg Rosette), teat wall thickness
1 cm proximal of the internal teat canal orifice