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## The mouthpiece chamber vacuum pattern indicates the cessation of milk flow and suits as an indicator to reduce teat end vacuum at a quarter level

Kathrin Schlappbach,<sup>1</sup> Momena Khatun,<sup>1,3</sup> Carl Oskar Paulrud,<sup>2</sup> and Rupert M. Bruckmaier<sup>1\*</sup>

<sup>1</sup>Veterinary Physiology, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland

<sup>2</sup>DeLaval SA, 14745 Tumba, Sweden

### ABSTRACT

We investigated the suitability of the quarter mouthpiece chamber vacuum (MPCV) as an indicator for cessation of quarter milk flow to potentially adjust the teat end vacuum at a quarter level. We tested the hypothesis that a MPCV increase is a clear indicator of quarter milk flow cessation. In addition, we tested if a quarter individual vacuum reduction at MPCV increase reduces the mechanical impact on the teat. Ten dairy cows were milked twice daily with a quarter specific vacuum supply with continuously high (51 kPa; TRT51) or low vacuum setting (41 kPa; TRT41), or high vacuum setting combined with a quarter specific vacuum reduction by 10 kPa immediately after the quarter specific MPCV increase (TRT51/41). Whole udder milk flow was continuously recorded. Each treatment was repeated at 4 subsequent milkings. The high vacuum settings (TRT51; TRT51/41) reached higher values in peak flow rate and average milk flow and consequently shorter machine-on time. The time from start of milking until the steep increase of the MPCV was shorter in front than rear quarters, and hence the time from start of MPCV increase until end of milking was longer in front than rear teats. Teat condition of the right front teats was measured for teat wall diameter by ultrasound and teat tissue thickness by cutimeter at 5 and 20 min after each experimental milking. The teat measurements were taken at the teat tip (distal barrel) and 2 cm above the teat tip (proximal barrel). The proximal teat wall diameter tended to be higher in TRT51 than in TRT41, both 5 and 20 min after milking. The distal teat wall diameter at 5 min was greater in TRT51 than in TRT41. In TRT51/41 the teat wall diameter at both locations was intermediate,

not significantly different from either TRT51 or TRT41. The distal teat tissue thickness was greater in TRT51 than in TRT41, and tended to be greater in TRT51/41 than in TRT41 at 5 min ( $P = 0.08$ ). The proximal teat tissue thickness at 5 min was higher in TRT51 and TRT51/41 than in TRT41. The teat tissue thickness decreased from 5 to 20 min only in the proximal barrel. The quarter individual MPCV increase appears to be a suitable indicator of the cessation of milk flow. The lack of a significant reduction of mechanical impact on the teat by a reduced vacuum of 41 kPa indicates that the vacuum level chosen may be still too high under conditions of a separate vacuum supply for each quarter which prevents a vacuum drop caused by the whole udder milk flow.

Keywords: mouthpiece chamber vacuum, teat condition, reduced vacuum, milk flow

### INTRODUCTION

The teat end vacuum changes during the course of milking and reaches levels almost as high as the system vacuum in the absence of the milk flow such as immediately after cluster attachment and at cessation of milk flow at the end of milking (Ambord and Bruckmaier, 2010). During milk flow, the vacuum drops proportionally to the milk flow level, the dimension of vacuum drop depending on the characteristics of the milk line (Besier and Bruckmaier, 2016). If the system vacuum remains unchanged during milking, its level must be set high enough to be still sufficient during the peak milk flow period despite the drop. In turn, an increased mechanical impact on the teat tissue occurs as soon as milk flow decreases and the high vacuum and respective liner movement acts on the empty teats at the end of milking (Odorcic et al., 2020). On the other hand, high milking vacuum leads to fast milk removal (Besier and Bruckmaier, 2016) as long as abundant milk is available through milk ejection during the plateau phase of milk flow. Potential risk factors on teat tissue integrity related to high vacuum are stretching of the teat during the liner-open phase of pulsation (Hamann

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<sup>3</sup>present address: Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

\*Correspondence: Prof. Rupert M. Bruckmaier, Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3012 Bern, Switzerland, Phone: +41-31-684 23 24, E-mail: [rupert.bruckmaier@vetsuisse.unibe.ch](mailto:rupert.bruckmaier@vetsuisse.unibe.ch)

and Mein, 1988), a high compressive load caused by the closed liner (Mein et al., 1987), and a particularly high mouthpiece chamber vacuum which develops once the respective udder quarter is close to be empty (Penry et al., 2017; Holst et al., 2021). Due to the anatomically determined distribution of milk storage with lower amount of milk in the front than in the rear quarters the front quarters are in most cows completely empty before the rear quarters (Rothenanger et al., 1995; Tančin et al., 2006). In contrast to automatic milking (Dzidic et al., 2004), a quarter-specific teat cup removal is currently not available in conventional milking systems. One vacuum source transmitted to all quarters by the claw, and consequently one long milk tube from the claw to the recorder, allows only the control of whole udder milk flow. Milking of empty teats (often classified as overmilking) can therefore not be avoided toward the end of milking when milk flow ceases in individual quarters decreasing and simultaneously the milk flow dependent vacuum drop disappears. A quarter specific teat cup detachment is obviously not possible in conventional milking clusters. However, a quarter specific vacuum reduction with keeping all teat cups attached if quarter milk flow ceases appears to be technically possible. A precondition for a technical solution is a reliable signal for the cessation of quarter milk flow and potentially to switch down the vacuum level in the respective quarters.

An increase of the mouthpiece chamber vacuum (MPCV) of each teat cup is expected to be an indicator of ceasing milk flow at a quarter level. The MPCV is a result of transferred vacuum from the claw to the MPC and the continuous air leaking through the mouthpiece liner lip. Albeit the same claw vacuum is acting on all quarters, the MPCV varies among quarters due to teat individual dimensions relative to the liner geometry (Penry et al., 2017; Holst et al., 2021). As soon as the intracisternal milk pressure in a teat lowers, the loss of seal and friction between the liner and the teat was shown to result in a quarter-specific steep increase of the MPCV (Mein et al., 1973a,b; Holst et al., 2021).

The primary goal of this study was to evaluate the suitability of the MPCV pattern as an indicator of quarter individual milk flow and its cessation. We tested the hypothesis that a characteristic increase of MPCV in each quarter toward the end of milking represents end of quarter milk flow. In addition, we investigated the effect of a 10 kPa vacuum reduction as soon as the MPCV increased toward the end of milk flow of individual quarters on milking performances and teat condition. A vacuum reduction at the end of milking has been previously shown to reduce the mechanical impact on the teats without a considerable

loss of milking performance (Rasmussen and Madsen, 2000; Stauffer et al., 2020).

## MATERIALS AND METHODS

All experimental procedures involving animals in this study followed the requirements of the Swiss animal protection and welfare law and were approved by the Veterinary Office of the Canton of Fribourg, Switzerland (authorization no. 2022\_01\_FR).

### *Animals and Housing*

Ten Holstein dairy cows from the Swiss Federal Research Station Agroscope Posieux were used in this study. At the start of the experimental period the cows were between 66 and 338 DIM of their first ( $n = 6$ ) or second ( $n = 4$ ) lactation. Quarter foremilk SCC was  $\leq 100,000$  cells/mL in all quarters, and needed to be free of signs of clinical mastitis throughout the study. Cows were kept in a tie stall barn, housed on straw and sawdust, with regular pasture access throughout the day. In the barn, cows were supplemented with TMR composed of grass silage, corn silage, hay and aftermath, as well as minerals and concentrate according to their individual production levels.

### *Experimental milkings and milk flow recording*

Because of the relatively long duration of experimental milkings including measurements of quarter milk yields and teat condition, experiments were conducted on 2 consecutive groups of 5 cows each. Experimental milkings were conducted twice daily in a tie stall at intervals of 12 h (0500, 1700), always in the same order to avoid irregular milking intervals and to allow similar conditions for all experimental milkings. Left-right alternating pulsation was provided at a pulsation rate of 60 cycles/min and a 65:35 pulsation rate by a pneumatic pulsator (Hydropuls HP 102, DeLaval, Tumba, Sweden).

Experimental milkings were performed with an especially constructed mobile quarter milking unit, which could be placed closely to the cow's udders to keep the milk tubes as short as possible. Experimental milkings were performed at 2 claw vacuum levels, provided from 2 different vacuum sources. The vacuum could be manually switched for each individual quarter between the 2 sources with a system vacuum of 44 or 53 kPa, respectively. The resulting claw vacuum level measured in one short milk tube (**SMT**) in the absence of milk flow were 41 and 51 kPa, respectively. The vacuum drop during the plateau phase of milk flow was only up to 2 kPa at a maximum because of the quarter-individual

transport of milk to the quarter-individual milk buckets (Figure 1).

To allow a quarter-individual vacuum supply and milk removal, a fully transparent quarter milking claw (formerly Surge-RX, Düringen, Switzerland, no longer available) was connected to the teat cups (Harmony, DeLaval, Tumba, Sweden) with round rubber liners (DeLaval product number 999007 03, mouthpiece liner lip diameter 20 mm, barrel diameter 22 mm, mouthpiece depth 44 mm). Four quarter milk tubes (3 m, inside diameter 1.4 cm) connected the cluster with 4 cylinder-shaped plastic buckets (Nalgene, 9.3L). The buckets were placed on a board, which was attached to a strain gauge electronic scales unit for whole udder milk flow recording as previously described (Rothenanger et al., 1995). The mass of the removed whole udder milk was continuously recorded, and the electronically differentiated signal (mass change every 2 s) allowed continuous recording of milk flow on a laptop computer. The cessation of milk flow in each quarter was additionally confirmed by visual observation of the 4 channels of the transparent claw.

Before each experimental milking a manual udder preparation was performed, consisting of removal of 3 squirts of foremilk from each quarter and subsequent teat cleaning with an alcohol-soaked gauze. The cluster was attached at 1 min after first touch of the udder (30 s udder preparation followed by a 30 s latency period). The cluster was detached manually after complete cessation of milk flow. Teat condition of the right front

quarter was measured at 5 min and 20 min after cluster detachment by ultrasound and cutimeter (details see below). Teat dipping in an iodine-based disinfectant was conducted immediately after the 20-min teat condition measurements.

Each quarter milk yield was determined by manual weighing each quarter bucket after milking to calculate the distribution of milk yield among quarters. Evaluated milking characteristics (Table 1) were total milk yield (**TMY**) as the sum of quarter yields, machine-on time (**MOT**) and peak flow rate (**PFR**, maximum milk flow maintained or surmounted for at least 20 s) measured by ruler from the obtained milk flow curves, and average milk flow (**AMF**, calculated by  $TMY/MOT$ ).

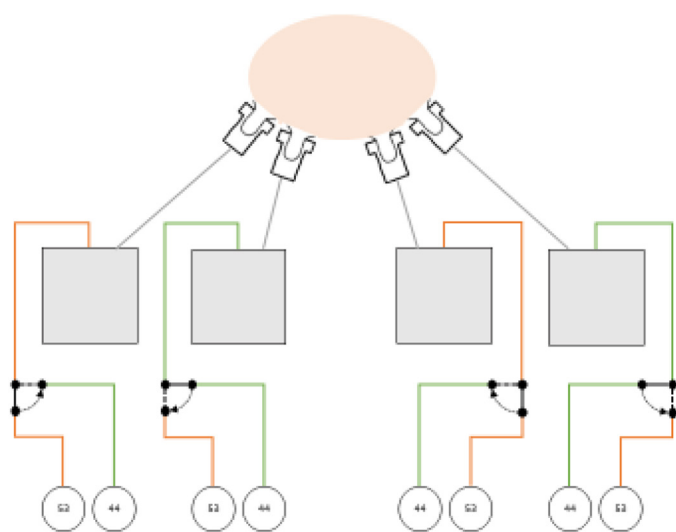
### Experimental Treatments

Each experimental cow was milked in each of the 3 treatments, and each treatment was conducted at 4 consecutive milkings (2 morning and 2 evening milkings) without a washout between treatments. The sequence of treatments was randomly assigned to each cow. Treatments were either standard claw vacuum (41 kPa) during entire milking (**TRT41**), high vacuum (51 kPa) during entire milking (**TRT51**), and high vacuum (51 kPa) until individual quarter vacuum switch to standard vacuum (41 kPa) after the quarter-specific increase of MPCV toward the end of milking which was considered to indicate cessation of milk flow of the respective quarter by visual observation in the claw (**TRT51/41**).

### Mouthpiece chamber vacuum recordings

During experimental milkings, the **MPCV** was simultaneously recorded in all 4 quarters. Two wireless portable pressure sensor devices (WPS, DeLaval) with 2 channels each were connected via silicone tubes (55cm, inside diameter 2 mm) to each of the 4 mouthpiece chambers (**MPC**). The MPCV data were real time transferred to a portable vacuum recording device (VPR200, DeLaval) to record the vacuum patterns at a frequency of 1 Hz. The real-time MPCV pattern during the ongoing milking enabled us to see the previously observed characteristic steep increase of MPCV when milk flow ceased in each quarter (Holst et al., 2021). A minimum MPCV increase of 5 kPa was used as threshold for the manual switch from the high to low vacuum in TRT51/41 in each quarter individually (see also Figures 1 and 2).

For further evaluation of the MPCV patterns selected the MPCV values at 20 s after attachment ( $V_{att}$ ), immediately before the steep increase ( $V_{inc}$ ), and at 20 s



**Figure 1.** : Diagram of the milking unit comprising 4 teat cups individually connected to 4 buckets. Each quarter milking unit is supplied by a vacuum source of either 44 kPa or 53 kPa according to the position of the switch (on/off), resulting in a teat end vacuum without milk flow of 41 kPa (TRT41), 51kPa (TRT 51), or both (TRT51/41) if switched after the MPCV increase.

**Table 1: Milking characteristics (mean  $\pm$  SEM) at different vacuum settings**

	Vacuum settings <sup>1</sup>		
	TRT41	TRT51	TRT51/41
Total milk yield (TMY, kg)	16.0 $\pm$ 0.2	16.2 $\pm$ 0.2	16.0 $\pm$ 0.2
Peak flow rate (PFR, kg/min)	4.7 $\pm$ 0.1 <sup>b</sup>	5.3 $\pm$ 0.1 <sup>a</sup>	5.5 $\pm$ 0.1 <sup>a</sup>
Average milk flow (AMF, kg/min)	3.1 $\pm$ 0.1 <sup>c</sup>	3.5 $\pm$ 0.1 <sup>a</sup>	3.3 $\pm$ 0.1 <sup>b</sup>
Machine on time (MOT, min)	5.5 $\pm$ 0.1 <sup>a</sup>	4.8 $\pm$ 0.1 <sup>b</sup>	5.0 $\pm$ 0.1 <sup>b</sup>

<sup>a-c</sup> Means with different lowercase letter differ significantly ( $P < 0.05$ ) between treatments within the same milking variable.

<sup>1</sup>Vacuum setting: TRT41 = low system vacuum (41 kPa); TRT51 = high system vacuum (51 kPa); TRT51/41 = high system vacuum with vacuum reduction (51 to 41 kPa) after quarter MPCV increase.

before cluster detachment ( $V_{det}$ ). In addition we determined for each quarter the time from start of milking until MPCV increase ( $t_{inc}$ ) and the time from MPCV increase until cluster detachment ( $t_{high}$ ). Results of MPCV are presented on Tables 2 and 3.

### Measurements of teat size and teat condition

The pre-milking teat size of all 4 teats of each cow was measured based on photographs in cranio-caudal direction of each teat, taken by a mobile phone camera (iPhone 6s). A transparent 2-dimensional reference scale was held at each teat while the picture was taken to enable later measurements of teat length and diameter at teat base and teat tip. Photographs were taken once for each cow shortly before routine evening milkings on days without experiments.

To record the short-term effects on teat tissue, teat wall diameter and teat tissue thickness of the right front teats were measured by ultrasound both pre- and post-milking. The pre-milking teat measurements were conducted at other than experimental milking days to avoid an impact of the teat handling on the milking characteristics. The post-milking measurements were taken at 5 and 20 min after cluster detachment at each experimental milking. Teat measurements were conducted close to the teat base, and 2 cm above the teat apex further named as proximal vs. the distal barrel, respectively. For transversal cross section scanning the teats were immersed in a plastic cup filled with hand warm water, and a 7 MHz convex probe connected to the ultrasound device (Draminski 4Vet; Draminski S.A., Olsztyn, Poland) was held to the outside of the cup by using contact gel (Lubricant Gel, Henry Schein, Melville, NY, USA) as previously described (Oodorčić et al., 2020; Stauffer et al., 2020). Measurements of the ultrasound images were conducted by using a graphic editing software (Adobe Photoshop, version 22.3.0, Adobe, San José, CA, USA). In addition, teat tissue thickness was measured by a spring-loaded caliper with

electronic display (Cutimeter; Hauptner-Herberholz, Solingen, Germany).

### Statistical analysis

For statistical analysis, RStudio Statistical Software (R version 4.1.2, <https://www.R-project.org/>) was used. All data are presented as arithmetic means  $\pm$  SEM. In the ANOVA model treatment and milking time (morning or evening) were included as fixed effects, and the interaction of milking time  $\times$  treatment was added. The individual cow included as repeated and random factor to consider for both repeated milkings and the 4 quarters of each udder. For whole udder milking characteristics and for MPCV characteristics and teat condition of the right front quarter the same model was used, however the cow only as a repeated factor. The F-test values of the fit linear mixed-effect model (lmer function in “lme4” package) was used to determine the overall effect of treatment, milking time and their interaction on milking characteristics, MPCV, and teat characteristics. There were no interactions between various factors, and interactions were therefore excluded from the final model. Significant treatment effects (F-test) and effects of quarters and udder halves were post hoc localized among the 3 treatments by Tukey’s  $t$ -test. Differences were considered significant if  $P < 0.05$ .  $P$ -values between 0.05 and 0.10 were considered as tendencies. Because the means of the MPCV characteristics did not differ among left and right quarters of both front and rear quarters MPCV data of left and right quarters were merged and presented as front and rear udder half means (Table 2). The MPCV characteristics of the right front teat are presented on Table 3 combined with teat condition measurements of the front right teat. Pearson correlation coefficients between teat dimensions and MPCV characteristics were calculated independent of treatment.

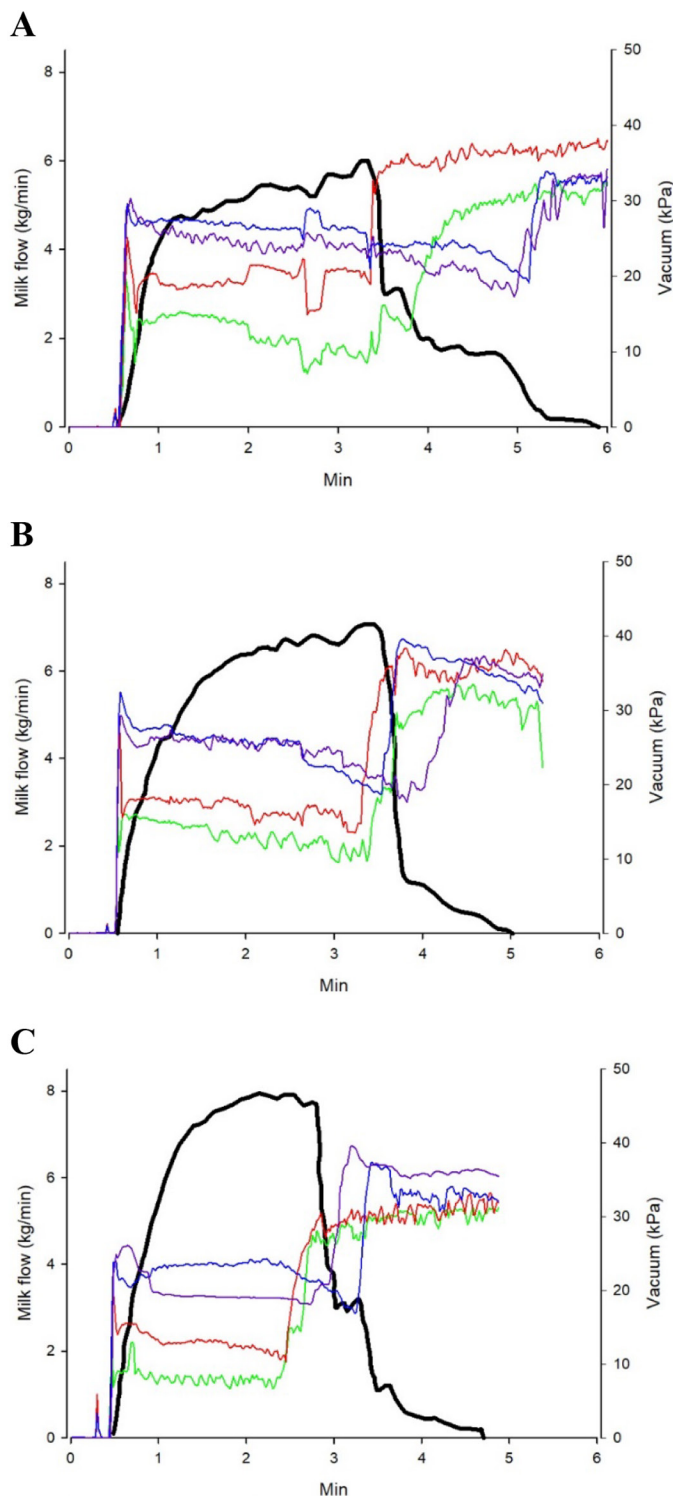
## RESULTS

## Milking characteristics

The TMY (Table 1) as well as the quarter milk yields (data not shown) did not differ among the 3 treatments. The milk yield was lower in front quarters ( $7.4 \pm 0.1$  kg;  $46 \pm 2\%$ ) than in rear quarters ( $8.8 \pm 0.1$  kg;  $54 \pm 2\%$ ) ( $P < 0.05$ ), similarly in all treatments. The PFR was higher in TRT51 and TRT51/41 than in TRT41 ( $P < 0.05$ ) (Table 1; Figure 2). The AMF differed among the treatments, with lowest values in TRT41 and highest values in TRT51 ( $P < 0.05$ ) (Table 1). The MOT was longer in TRT41 than in TRT51 and TRT51/41 ( $P < 0.05$ ) but not significantly different between TRT 51 and TRT51/41 (Table 1; Figure 2).

## Mouthpiece chamber vacuum patterns

Front and rear udder half means and the right front teat measurements separately are presented on Tables 2 and 3, respectively. In all quarters and treatments, the MPCV was considerably lower than the system vacuum throughout milking. Despite different vacuum levels (41 or 51 kPa)  $V_{att}$  did not differ among treatments ( $P = 0.28$ ) (Table 2; Figure 2), but was generally higher in rear than in front quarters ( $P < 0.05$ ). During the further course of milking, the MPCV decreased significantly in all quarters and treatments until  $V_{inc}$  ( $P < 0.05$ ) except for TRT51/41 in front quarters ( $P = 0.25$ ), and remained lower in front than rear quarters until the occurrence of the quarter specific steep increase toward the end of milk flow (Table 2; Figure 2). The MPCV increase occurred individually in each quarter which was related to the quarter-individual dramatic decline and cessation of milk flow (visually observed in the claw), and reached highest levels at  $V_{det}$ , similar in front and rear quarters, but higher in TRT51 than TRT41 and TRT51/41 ( $P < 0.05$ ). Also the MPCV in the separately recorded right front quarter (the quarter where teat condition measurements were performed) reached the levels at 20 s before detachment ( $V_{det}$ ) with higher values in TRT51 than in TRT41 and TRT51/41 ( $P < 0.05$ ; Table 3). The  $t_{inc}$  was longer in TRT41 than TRT51 and TRT51/41 and was longer in rear than front quarters ( $P < 0.05$ ) (Table 2; Figure 2). Accordingly, the  $t_{inc}$  of the right front quarter was longer in TRT41 than TRT51 and TRT51/41 (Table 3). Consequently,  $t_{high}$  was significantly longer in the front udder half compared with the rear udder half. Only in the rear udder half  $t_{high}$  was longer in TRT51/41 than in TRT41 and TRT51 ( $P < 0.05$ ). Accordingly, the  $t_{high}$  of the right front quarter did not differ among treatments (Table 3).



**Figure 2.** : Example milk flow curves and quarter mouthpiece chamber vacuum in treatments: A = TRT41, B = TRT51, C = TRT51/41. Mf = milk flow; MPCV = mouthpiece chamber vacuum (green = front right; red = front left; violet = rear right; blue = rear left).

**Table 2: Mouthpiece chamber vacuum characteristics of front vs. rear quarters (mean  $\pm$  SEM) at different vacuum settings**

MPCV <sup>2</sup>	Vacuum settings <sup>1</sup>					
	Front quarters			Rear quarters		
	TRT41	TRT51	TRT51/41	TRT41	TRT51	TRT51/41
V <sub>att</sub> (kPa)	14.8 $\pm$ 1.0 <sup>*B</sup>	14.6 $\pm$ 1.0 <sup>*B</sup>	13.7 $\pm$ 1.0 <sup>*B</sup>	25.7 $\pm$ 1.1 <sup>B</sup>	26.0 $\pm$ 1.3 <sup>B</sup>	24.7 $\pm$ 1.2 <sup>B</sup>
V <sub>inc</sub> (kPa)	11.8 $\pm$ 0.6 <sup>*C</sup>	12.2 $\pm$ 0.6 <sup>*C</sup>	12.5 $\pm$ 0.6 <sup>*B</sup>	16.7 $\pm$ 0.7 <sup>C</sup>	17.4 $\pm$ 0.6 <sup>C</sup>	17.4 $\pm$ 0.7 <sup>C</sup>
V <sub>det</sub> (kPa)	33.4 $\pm$ 0.5 <sup>bA</sup>	35.4 $\pm$ 0.5 <sup>bA</sup>	33.6 $\pm$ 0.4 <sup>bA</sup>	32.4 $\pm$ 0.5 <sup>BA</sup>	36.1 $\pm$ 0.6 <sup>AA</sup>	33.3 $\pm$ 0.5 <sup>BA</sup>
t <sub>inc</sub> (min)	3.4 $\pm$ 0.1 <sup>a*</sup>	2.8 $\pm$ 0.1 <sup>b*</sup>	2.8 $\pm$ 0.1 <sup>b*</sup>	4.2 $\pm$ 0.2 <sup>A</sup>	3.5 $\pm$ 0.2 <sup>B</sup>	3.4 $\pm$ 0.1 <sup>B</sup>
t <sub>high</sub> (min)	2.0 $\pm$ 0.1 <sup>*</sup>	2.0 $\pm$ 0.1 <sup>*</sup>	2.2 $\pm$ 0.1 <sup>*</sup>	1.2 $\pm$ 0.1 <sup>B</sup>	1.3 $\pm$ 0.1 <sup>B</sup>	1.6 $\pm$ 0.1 <sup>A</sup>

<sup>a,b</sup>Means with different lowercase letter differ significantly ( $P < 0.05$ ) between treatments within front quarters.

<sup>A,B</sup>Means with different uppercase letter differ significantly ( $P < 0.05$ ) between treatments within rear quarters.

<sup>A-C</sup>Means with different uppercase letter differ significantly ( $P < 0.05$ ) within treatments within front or rear quarters, respectively.

\* Means of front quarters differ significantly ( $P < 0.05$ ) between respective means of rear quarters.

<sup>1</sup>System vacuum setting: TRT41 = low system vacuum (41 kPa); TRT51 = high system vacuum (51 kPa); TRT51/41 = high system vacuum with vacuum reduction (51 to 41 kPa) after quarter MPCV increase.

<sup>2</sup>Mouthpiece chamber vacuum: V<sub>att</sub> = MPCV 20 s after attachment; V<sub>inc</sub> = MPCV level before increase; V<sub>det</sub> = MPCV 20 s before detachment; t<sub>inc</sub> = time point at MPCV increase; t<sub>high</sub> = time from start of MPCV increase until end of milking.

In all 3 treatments the last quarter to reach t<sub>inc</sub> was at most milkings one of the rear quarters (TRT41 72%; TRT51 87%; TRT51/41 79%). The time span between the first and the last quarter MPCV increase within one milking, i.e., the time of a potential vacuum reduction being active in individual quarters, did not significantly differ among treatments and was 1.5  $\pm$  0.1 min in TRT41, 1.3  $\pm$  0.1 min in TRT51, and 1.3  $\pm$  0.1 min TRT51/41.

### Pre- and post-milking teat condition

Pre-milking length of the front teats, analyzed from the teat photographs, was significantly greater than the length of the rear teats (4.5  $\pm$  0.5 cm vs. 3.9  $\pm$  0.1 cm,  $P < 0.05$ ). However, the teat diameter did not differ among front and rear teats. The teat length was negatively correlated with the V<sub>att</sub> ( $r = -0.31$ ,  $P < 0.05$ ), V<sub>inc</sub> ( $r = -0.17$ ,  $P < 0.05$ ) and V<sub>det</sub> ( $r = -0.18$ ,  $P$

**Table 3: Mouthpiece chamber vacuum and teat characteristics (means  $\pm$  SEM) of right front quarter at different vacuum settings**

MPCV patterns <sup>2</sup>	Vacuum settings <sup>1</sup>	Vacuum settings <sup>1</sup>		
		TRT41	TRT51	TRT51/41
V <sub>att</sub> (kPa)		13.8 $\pm$ 1.4 <sup>B</sup>	13.6 $\pm$ 1.3 <sup>B</sup>	13.0 $\pm$ 1.2 <sup>B</sup>
V <sub>inc</sub> (kPa)		11.9 $\pm$ 0.8 <sup>B</sup>	11.7 $\pm$ 0.8 <sup>B</sup>	11.6 $\pm$ 0.7 <sup>B</sup>
V <sub>det</sub> (kPa)		32.5 $\pm$ 0.7 <sup>bA</sup>	35.1 $\pm$ 0.8 <sup>bA</sup>	33.0 $\pm$ 0.6 <sup>bA</sup>
t <sub>inc</sub> (min)		3.3 $\pm$ 0.1 <sup>a</sup>	2.8 $\pm$ 0.1 <sup>b</sup>	2.7 $\pm$ 0.1 <sup>b</sup>
t <sub>high</sub> (min)		2.1 $\pm$ 0.2	2.1 $\pm$ 0.1	2.3 $\pm$ 0.1
Teat wall diameter <sup>3</sup> (distal barrel)	At 5 min (mm)	8.2 $\pm$ 0.2 <sup>b</sup>	8.8 $\pm$ 0.2 <sup>a</sup>	8.4 $\pm$ 0.3 <sup>*ab</sup>
	At 20 min (mm)	7.5 $\pm$ 0.2	7.9 $\pm$ 0.2	7.6 $\pm$ 0.2
Teat wall diameter <sup>3</sup> (proximal barrel)	At 5 min (mm)	10.0 $\pm$ 0.4 <sup>*</sup>	11.0 $\pm$ 0.4 <sup>(*)*</sup>	10.8 $\pm$ 0.4 <sup>*</sup>
	At 20 min (mm)	8.0 $\pm$ 0.2	8.7 $\pm$ 0.3 <sup>(*)</sup>	8.5 $\pm$ 0.3
Teat tissue thickness <sup>3</sup> (distal barrel)	At 5 min (mm)	11.0 $\pm$ 0.2 <sup>b</sup>	11.7 $\pm$ 0.3 <sup>a</sup>	11.5 $\pm$ 0.2 <sup>ab (*)</sup>
	At 20 min (mm)	10.7 $\pm$ 0.2 <sup>b</sup>	11.4 $\pm$ 0.2 <sup>a</sup>	11.0 $\pm$ 0.2 <sup>ab</sup>
Teat tissue thickness <sup>3</sup> (proximal barrel)	At 5 min (mm)	12.2 $\pm$ 0.3 <sup>b*</sup>	13.5 $\pm$ 0.3 <sup>a*</sup>	13.4 $\pm$ 0.4 <sup>a*</sup>
	At 20 min (mm)	11.2 $\pm$ 0.3	11.8 $\pm$ 0.3	11.7 $\pm$ 0.3

<sup>a,b</sup>Means with different lowercase letter differ significantly ( $P < 0.05$ ) between treatments.. <sup>A,B</sup>Means with different uppercase letter differ significantly ( $P < 0.05$ ) within treatments.

\* Means differ significantly ( $P < 0.05$ ) between 5 and 20 min after milking within the same treatment.

(\*)Means tend to differ ( $P = 0.05 - 0.1$ ) between the respective TRT41 teat condition measurement.

<sup>1</sup>Vacuum setting: TRT41 = low system vacuum (41 kPa); TRT51 = high system vacuum (51 kPa); TRT51/41 = high system vacuum with vacuum reduction (51 to 41 kPa) after quarter MPCV increase.

<sup>2</sup>Mouthpiece chamber vacuum: V<sub>att</sub> = MPCV 20 s after attachment; V<sub>inc</sub> = MPCV level before increase; V<sub>det</sub> = MPCV 20 s before detachment; t<sub>inc</sub> = time point at MPCV increase; t<sub>high</sub> = time from start of MPCV increase until end of milking.

<sup>3</sup>Teat characteristics: teat tissue thickness (measured by cutimeter) and teat wall diameter (transversal images measured by B - mode ultrasound)

< 0.05), i.e., the MPCV throughout milking was lower in long than in short teats. With interpreting these correlation coefficients it needs to be considered that the teat length did almost not vary in rear teats, and only slightly in front teats. Therefore, teat lengths was confounding with the udder position and with the milk yield of the respective quarters.

The pre-milking teat wall diameter of the right front teat, measured by ultrasound, was  $6.4 \pm 0.3$  mm at proximal barrel and  $6.1 \pm 0.5$  mm at distal barrel. Both at proximal and distal barrel teat wall diameter was greater at 5 and 20 min after milking compared with pre-milking values ( $P < 0.05$ ), although the teat wall diameter decreased from 5 to 20 min ( $P < 0.05$ ). The teat wall diameter at the distal barrel at 5 min after milking was greater in TRT51 than TRT41 (Table 3;  $P < 0.05$ ), and at the proximal barrel the teat wall diameter tended to be greater in TRT51 than in TRT41 both at 5 min ( $P = 0.073$ ) and at 20 min after milking ( $P = 0.066$ ).

The pre-milking teat tissue thickness of the right front teat, measured by cutimeter, was  $11.3 \pm 0.4$  mm at proximal barrel and  $10.7 \pm 0.3$  mm at the distal barrel. At 5 min after milking, teat tissue thickness both at both proximal and distal barrel was greater than before milking in all treatments ( $P < 0.05$ ), whereas teat tissue thickness there was no more difference of between before and 20 min after milking at the proximal barrel. From 5 to 20 min after milking the teat tissue thickness decreased significantly in all treatments ( $P < 0.05$ ). At 5 min after milking, the teat tissue thickness at the proximal barrel was greater in TRT51 and TRT51/41 than in TRT41 ( $P < 0.05$ ), and at the distal barrel the teat tissue thickness was greater in TRT51 than TRT41 ( $P < 0.05$ ), and tended to be greater in TRT51/41 than in TRT41 ( $P = 0.089$ ). At 20 min after milking, the distal teat tissue thickness was still greater in TRT51 than in TRT41.

## DISCUSSION

Milking performance has previously been shown to be improved by milking at 50 kPa compared with 42 kPa, however at an increased mechanical impact on the teat tissue (Besier and Bruckmaier, 2016). In a later study, the impact of high vacuum on the teats could be reduced if the vacuum was switched to a lower level during periods of low milk flow at the start and end of milking at a whole udder level, importantly without a considerable loss of milking performance (Stauffer et al., 2020). It is obvious that the mechanical impact on the teat tissue causing venous congestion and tissue swelling (Stauffer et al., 2021) occurs toward the end of milking, and is mainly due to high vacuum and si-

multaneously low filling of the teat with milk (Odorcic et al., 2020). Therefore, the present study investigated a vacuum reduction at an individual quarter level to particularly consider the reduced filling of the teat of individual quarters. As an indicator of inadequate teat filling and cessation of milk flow in individual quarters we tested the suitability of the MPCV and its characteristic increase when the seal between the teat and the liner barrel gets lost. The vacuum at the teat end (claw vacuum, measured in one SMT) in conventional clusters is subject to milk flow dependent drops and fluctuations, but similar in all 4 quarters. It has to be considered that this is not the case in the present experimental setup because through the used quarter milking claw each quarter was provided with an own vacuum source. Consequently, the teat end vacuum was not necessarily similar in all quarters, and there was only a slight vacuum drop (2–3 kPa, measured not at all experimental milkings) during the plateau phase of milking flow. Differently to the teat end vacuum in conventional milking systems, previous work has demonstrated that the MPCV increases considerably in individual quarters as soon as the seal between the teat and the liner is no longer intact, which occurs concomitantly with a dramatic decrease shortly before a complete cessation of the respective quarter milk flow (Mein et al., 1973b; Borkhus and Rønningen, 2003; Holst et al., 2021). Thus, the characteristic increase of MPCV is also supposed to be a marker for the start of increased mechanical impact on the teat tissue because increased stretching and compression of the soft teats walls during open and closed liner, respectively. In addition to being a marker, the elevated MPCV may cause an additional stretching of the tissue at the level of the MPC, i.e., close to the teat basis.

As expected and demonstrated in previous studies the milk yield, i.e., the degree of udder emptying, was not affected by the used vacuum levels and did therefore not differ among treatments. Also the distribution of milk yield in front and rear quarters with less milk in front than in rear quarters confirms earlier findings of higher milk production in the rear than in front quarters (Rothenanger et al., 1995; Tančin et al., 2006; Penry et al., 2018). The similarly higher PFR and shorter MOT in TRT51 and TRT51/41 compared with TRT41 demonstrates that the vacuum reduction at the end of milk flow of individual quarters in TRT51/41 did not considerably reduce the milking performance. This finding was also supported by the higher AMF in TRT51/41 compared with TRT41 albeit the highest AMF was achieved in TRT51. The almost similar milking performance in both TRT51 and TRT51/41, however higher than in TRT41, demonstrates that the decreased teat end vacuum after the increase of MPCV

did no longer affect the milk flow because the quarter was at least almost emptied when the vacuum was switched to the lower level.

At any time during milking, the MPCV was considerably lower than the teat end vacuum in all quarters. This observation demonstrated that the vacuum to the MPC is transferred from the claw and short milk tubes via the teat end. Due to the contact between teat and liner and simultaneously continuous air leakage through the mouthpiece liner lip, there was a continuous loss of vacuum in the MPC (Holst et al., 2021). During high milk flow, the MPCV was low because of the positive milk pressure in the teat cistern leading to a tight seal and friction between the teat and the liner barrel (Mein et al., 1973b). Already during the first seconds after teat cup attachment we observed a fast decline of MPCV indicating an optimal seal between the teat and the liner. In a previous study we have demonstrated that this is particularly the case if round liners are used for milking (Holst et al., 2021). The respective MPCV measurements at 20 s of milking ( $V_{att}$ ) did not differ among treatments despite a 10 kPa difference in the teat end vacuum. Likely, the transfer of vacuum from the teat end to the MPC during the liner-open phases was higher at 51 kPa compared with 41 kPa. If so, this difference was completely compensated by an also higher air leakage between the teat and the mouthpiece liner lip at the higher vacuum level. The course of the MPCV continued mostly by a further gradual but flat decline during the first minutes of milking which may be caused by the recently detected moderate further increase of cisternal size and hence increased intracisternal milk pressure and adhesion between teat and liner (Tuor et al., 2023). Lowest MPCV levels were reached immediately before the steep increase of MPCV ( $V_{inc}$ ) likely caused by a considerable loss of the seal between the teat and liner because the teat was no longer filled with milk during the liner-closed phase of pulsation. This time point indicated the cessation of milk flow in the respective quarter (based on visual observation in the 4 transparent and clearly visible chambers of the used claw). Like the  $V_{att}$  also the  $V_{inc}$  did not differ among treatments despite still different vacuum levels at the teat end. Likely a slightly different transfer of vacuum into the MPC at different vacuum settings is fully compensated by the leaking liner lip (Mein et al., 1973a, b) which was obviously lower in rear than in front teats. Because the rear teats were shorter than the front teats, the lip was likely closer to the teat base which may have caused an additional seal between the lip and the gland tissue (Vierbauch et al., 2021; Weiss et al., 2004). The teat diameter at the teat base did not differ among front and rear teats.

Only the high MPCV measured immediately before cluster detachment ( $V_{det}$  was higher in TRT51 compared with the 2 other treatments where the claw vacuum was 41 kPa. Thus, only after the seal between teat and liner was completely gone due to the lack of milk refill in the teat, the MPCV was much more affected by the teat end vacuum level than by the mouthpiece liner lip leakage before. At this point there was also no longer a difference between front and rear teats which indicates that either teat length or udder position did no longer play a role to control the transfer of vacuum from the teat end to the MPC in the teat which are no longer filled with milk.

The time of MPCV increase ( $t_{inc}$ ) occurred earlier at 51 kPa (TRT51; TRT51/41) than at 41 kPa (TRT41), which was due to the higher milk flow (AMF) and hence earlier emptying of all quarters at high than at low vacuum causing an earlier emptying of the respective quarters. Because of the lower milk yield of front than rear quarters (Rothenanger et al., 1995) and hence shorter duration of milk flow, the  $t_{inc}$  occurred earlier in front than in rear quarters.

Consequently and because the milking unit was detached simultaneously for all quarters, the time of elevated MPCV ( $t_{high}$ ) was longer in front than in rear quarters. Thus, the vacuum reduction from  $t_{inc}$  until cluster detachment in TRT51/41 with its expected reduced mechanical impact on the teat tissue was longer effective in front than rear quarters. The steep increase of MPCV demonstrates the expected reduced adhesion between the teat and the liner, which was caused by the lack of teat cistern refill during the phases of closed liner. At this particular point in milking, when milk flow ceased and the teat end vacuum gradually increased (not measured in the present study) the mechanical load on teat tissue increased as documented in earlier studies (Isaksson and Lind, 1992; Odorčić et al., 2019; 2020). A reduction of the milking intensity either by reduced vacuum or shorter liner-open phases is recommended to minimize the mechanical load on the teat, and to harvest the remaining small amount of milk, if any is still in the udder. Anyway, in conventional milking systems, it is not possible to detach teat cups quarter individually but adjustments in the vacuum (Tuor et al., 2021; Stauffer et al., 2020) and/or pulsation settings are possible solutions to reduce the mechanical load on teat tissue toward the end of milking.

### Teat condition

Relative to pre-milking measurements the teat tissue thickness was increased at 5 min after milking at the proximal barrel in all treatments, and in the distal



barrel only in TRT51 and TRT51/41. At 20 min after milking teat tissue thickness was partially recovered at both locations in all treatments except for TRT51 where values remained significantly elevated at the distal barrel. In contrast to cutimeter measurements ultrasound teat wall diameter was increased at 5 min after milking in all treatments and remained increased also at 20 min. This effect can partially also be caused by the low intracisternal pressure which leads to a higher teat wall diameter also without a particular mechanical load (Odorčić et al., 2020). In contrast, the cutimeter can measure the teat tissue thickness without being affected by higher or lower milk pressure in the teat cistern and therefore this method is more sensitive than ultrasound for recording of teat tissue recovery (Odorčić et al., 2020).

Highest values of both teat tissue thickness and teat wall diameter were achieved in TRT51, however not significantly different from TRT51/41. Only in TRT41 the increase of teat tissue thickness was less pronounced which indicates the entire milking process at lower vacuum had a lower impact on the teat tissue than milking at high vacuum. Therefore, the vacuum reduction from 51 to 41 kPa only after MPCV increase at the end of milking in TRT51/41 was not fully compensating for the higher mechanical impact through the 51 kPa vacuum before. A partial recovery of the teat tissue thickness occurred in all treatments however only at the proximal and not the distal barrel whereas the teat wall diameter showed a partial recovery at both locations. This may indicate that the impact of vacuum had a stronger effect on the tissue at the teat base.

## CONCLUSION

Milking at high vacuum has the highest milking performances but also the highest impact on teat tissue. The vacuum reduction after the MPCV did only show tendencies of a reduced impact on the teat tissue. However, the vacuum reduction did not cause a reduction of milking performance compared with continuously high vacuum. The vacuum reduction in TRT51/41 may have been too short to show a significant effect if the continuous high vacuum and absence of vacuum drop in the specific experimental setup is considered. The MPCV increase at a quarter level is a suitable indicator of cessation of quarter milk flow. Given the availability of a technical solution this could be used as a signal for vacuum reduction at a quarter level in milking systems where a quarter individual teat cup detachment is not possible.

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## ORCID

Carl Oskar Paulrud  <https://orcid.org/0000-0002-2772-8064>  
Rupert M. Bruckmaier  <https://orcid.org/0000-0002-9374-5890>