



The interplay of continuous milk ejection and milking system with and without prestimulation at different vacuum settings

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ABSTRACT

Efficient machine milking requires an optimal interaction of alveolar milk ejection in the udder and milk removal by the milking machine. The aim of the present study was to test whether the equilibrium between continuous milk ejection and milk removal can also be maintained at very fast milking through a particularly high vacuum. Eight Holstein dairy cows were milked at 42, 52, or 60 kPa, with (PS) or without (nPS) prestimulation. Each of the 6 treatments was conducted at 2 afternoon milkings in each animal. The prestimulation lasted 40 s and consisted of forestripping and teat cleaning. The cluster attachment followed after a 20-s latency period. Throughout each milking, B-mode ultrasound videos of the gland cistern of 1 front quarter as well as milk flow and claw vacuum curves were recorded. Total milk yield was neither affected by nPS or PS nor by the vacuum level. Milk removed within the first minute and the first 2 min of milking and average milk flow were higher, and the duration of incline and time until peak milk flow were shorter at PS than at nPS milkings at all vacuum levels. Machine-on time was shorter at PS than at nPS milkings, although only at 42 and 52 kPa vacuum, obviously caused by the high percentage of bimodalities occurring in nPS milkings (17% bimodalities in PS vs. 92% bimodalities in nPS milkings). The frequency of bimodalities was higher at high than at low vacuum both in PS and nPS milkings. Peak flow rate and average milk flow were both higher at higher vacuum levels. The duration of milk flow plateau was shorter at 60 kPa than at 42 kPa milkings. At the highest vacuum (60 kPa), the shorter plateau phase indicated a declining milk ejection rate toward the end of the plateau phase, and milk ejection could no longer keep up with the fast milk removal; hence, a higher milking efficiency at a higher vacuum level could only be achieved as long as the gland cistern remained sufficiently filled by the continuous milk ejection.

The ultrasound imaging confirmed this finding as the duration of cisternal area plateau in the recorded front quarter was shorter at high than at low vacuum. Thus, the highest vacuum of 60 kPa did not cause a shorter machine-on time than 52 kPa. In conclusion, milking at a very high vacuum can increase milking efficiency compared with a low vacuum. However, a vacuum reduction at the start and toward the end of milking is required to prevent overmilking if milking is performed at a very high vacuum.

Key words: prestimulation, ultrasound, bimodality, vacuum level

INTRODUCTION

Milk within the udder can be divided into the following 2 fractions: cisternal and alveolar milk. The cisternal fraction is available before oxytocin is released from the posterior pituitary gland; hence, milk ejection occurs (Knight et al., 1994; Pfeilsticker et al., 1996; Bruckmaier and Blum, 1998). Prestimulation induces an oxytocin release and, hence, a contraction of myoepithelial cells to shift the milk from the alveolar tissue into the gland cisterns of each quarter already before the start of milk removal. Depending on the degree of udder filling, it takes about 1 min from the start of prestimulation until milk ejection occurs (Bruckmaier and Hilger, 2001). A lacking or insufficient prestimulation results in delayed milk ejection and, hence, a separate removal of the cisternal milk before milk ejection, which causes a bimodal milk flow pattern (Kaskous and Bruckmaier, 2011; Vetter et al., 2014; Tuor et al., 2022). In addition to manual or mechanical prestimulation, the rhythmic movement of the teat cup liners continues the teat stimulation throughout milking. Thus, oxytocin is released throughout milking, which is required for continuous milk ejection and complete udder emptying (Bruckmaier et al., 1994).

The use of B-mode ultrasound imaging has been shown to be an excellent tool to evaluate teat condition (Besier and Bruckmaier, 2016; Odorcic et al., 2020; Stauffer et al., 2020), to estimate milk partitioning in the udder, and to record morphologic changes in the

Received August 16, 2022.

Accepted December 10, 2022.

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udder cistern (Bruckmaier and Blum, 1992; Ayadi et al., 2003; Caja et al., 2004). It has been possible to visualize alveolar milk ejection in response to oxytocin as an enlargement of the gland cistern by ultrasound imaging (Bruckmaier and Blum, 1992). Based on that, we assumed that continuous imaging of the gland cistern is also suitable to visualize the interaction of continuous cisternal fill and milk removal throughout the course of milking. An earlier study indicated that milking at a very high milking intensity (e.g., by prolonged b-phase of pulsation) leads to a faster cisternal emptying than cisternal refill by milk ejection as indicated by reduced intracisternal milk pressure (Pfeilsticker et al., 1995). By using B-mode ultrasound during milking, we wanted to visualize the interplay of milk removal by the milking vacuum and the concomitant continuous shift of alveolar milk into the cistern with expectedly constant size of the cistern until the refill of alveolar milk slows down when the quarter is emptied. The present work was performed to test whether cisternal ultrasound is suitable to visualize the presence and loss of the equilibrium of milk ejection and milk removal depending on the intensity of milk extraction. We hypothesized that the faster milk removal at high compared with low vacuum would cause an earlier decline of cisternal size and milk flow because the cisternal refill cannot keep pace with the high speed of milk removal. In addition, we intended to visualize the effect of milking without prestimulation on the cisternal size before and during milk ejection.

MATERIALS AND METHODS

The experiments of this study were conducted in compliance with the requirements of the Swiss animal protection and welfare law and were authorized by the Veterinary Office of the Canton of Fribourg (authorization no 2020_40_FR).

Animals and Housing

Eight Holstein dairy cows from the Swiss Federal Research Station Agroscope Posieux were used. At the start of experimental milkings, cows were between 6 and 236 DIM of their second to fifth lactation [second ($n = 1$), third ($n = 2$), and fifth ($n = 5$)]. The 305-d milk production of the experimental cows in their previous lactation ranged between 7,321 and 10,410 kg (mean $9,127 \pm 442$ kg SEM). All cows passed a general health check before the experiments and were free of signs of clinical mastitis. The cows were kept in loose housing between milkings, and experimental milkings were performed in a tiestall. Cows were fed a TMR

of corn silage, grass silage, and aftermath, which was supplemented by minerals as well as concentrate according to their individual production levels after each milking.

Milking Equipment and Treatments

Experimental milkings were performed in 2 groups (4 cows each) at 1600 h. Morning milkings were performed at 0500 h according to the usual routine of the farm, which included prestripping and short udder preparation before cluster attachment. Morning milkings served as “washout” from the previous experimental milking. The cows were milked with a bucket milker. The milking cluster consisted of a top flow claw (Harmony, DeLaval) and teat cups with round rubber liners (product number 99900901, DeLaval). The pulsation rate was 60 cycles per minute and the pulsation ratio was 65/35 in all treatments. Milkings were performed either with or without prestimulation (PS = with prestimulation, nPS = no prestimulation) and at system vacuum levels of 42, 52, or 60 kPa, resulting in 6 different treatments. For each cow, each treatment was performed at 2 subsequent afternoon milkings, although at an individual-cow randomized sequence to exclude treatment \times test day confounding. Prestimulation lasted 40 s and included forestripping into a cup (2–3 squirts per teat) and teat cleaning using disposable disinfectant cloths. The cluster was attached after a 20-s latency period. At nPS milkings, the cluster was attached without any previous touch such as teat cleaning or prestrip.

Milk Flow Recording and Milking Characteristics

Milk flow was recorded during all experimental milkings using a portable electronic recording unit (LactoCorder, WMB AG). The following milking characteristics were analyzed using the LactoPro software (version 6.0.60, WMB AG): total milk yield (**TMY**), milk yield milked in the first (**MY1**) and the first 2 min of milking (**MY2**), duration of incline (**dI**; time period between milk flow over 500 g/min or resurgence of milk flow after bimodality until plateau phase), duration of plateau (**dP**; time period between threshold slopes of <0.8 and >0.8 kg/min² milk flow), time until peak flow rate (**tPFR**), peak flow rate (**PFR**; maximum milk flow maintained for at least 22.4 s), duration of decline (time period after plateau phase until cluster detachment), machine-on time (**MOT**), and average milk flow (**AMF**; calculated by TMY/MOT). Furthermore, we calculated the percentages of bimodal milk curves using the definition of bimodal milk curve of the LactoPro software. A milk flow curve was defined as bimodal

if the milk flow declined more than 200 g/min after a milk flow of at least 500 g/min in the first 96 s was reached.

Vacuum Measurements and Ultrasound Imaging of One Gland Cistern

Claw vacuum levels were recorded throughout all experimental milkings via a cannula in one of the short milk tubes by using a portable vacuum reader (VPR200, DeLaval) connected to a wireless sensor (WPS, DeLaval). The system vacuum levels 42, 52, and 60 kPa at the vacuum pump resulted in a claw vacuum in the absence of milk flow of 40, 48, and 56 kPa, respectively.

The B-mode ultrasound images of the gland cistern of 1 front quarter were recorded during the whole milking using a convex probe (Draminski 4Vet, Draminski S.A.; 2 to 8 MHz, radius 50 mm, penetration depth 150 mm). Before the start of the trial, either the left or right front quarter was selected of each cow, depending on its better suitability for ultrasound imaging. Using contact gel (Lubricant Gel, Henry Schein), the probe was placed on the respective quarter right at the teat base. Ultrasound imaging started only immediately after cluster attachment to avoid an effect on milk ejection by the placement of the ultrasound probe mainly at nPS milkings. For analysis the ultrasound videos were converted into individual pictures (1 picture per second) using a converting software (Free Video to JPG Converter, version 5.0.101, Digital Wave LTD) and the area of the nonechoic gland cistern (recognized by different markers of the tissue) was measured every 20 s using the graphic editing software Photoshop (version 22.3.0, Adobe). To better perceive changes of the size of the area, measurements were conducted every 5 s at periods when the size of the area seemed to get larger or smaller (e.g., if a bimodality occurred). The measured size in pixels was converted into cm^2 . To analyze the changes of the cisternal area during the course of milking, we defined the following stages of cisternal size. The maximum area (cm^2) was defined as the largest area during milking. After teat cup attachment, the cisternal area increased at all milkings and we defined duration of area increase (**dAI**, min) until the area went into a steady state (duration of area plateau, **dAP**, min). The end of the area plateau was defined as time until the size of the cistern started to decrease after peak milk flow (**tEAP**, min). At first, the cisternal area started to decrease slowly (duration of slow area decrease, **dSAD**, min), followed by a fast reduction of the area size (duration of fast area decrease, **dFAD**, min). The time point when the area did

not decrease anymore or disappeared was defined as the end of area decrease (**EAD**, min).

Statistical Analysis

For statistical analysis, we used the SAS software (version 9.4, SAS Institute Inc.). The data are presented as arithmetic means and standard errors of the respective means. Statistical testing was performed based on LSMEANS using the MIXED procedure of SAS. The model included the prestimulation (PS or nPS) and the system vacuum as fixed effects as well as the interaction between the 2 variables (which was not significant for any of the evaluated parameters). The cow was included in the model as a repeated and random factor. The Tukey-Kramer test was used to localize differences, which were considered as significant if $P < 0.05$. At $0.05 \leq P < 0.10$ differences were considered as tendencies.

RESULTS

Milking Characteristics and Vacuum Measurements

The TMY neither differed among vacuum levels nor between PS and nPS milkings (Table 1). Both MY1 and MY2 were higher at PS than nPS milkings at all vacuum levels ($P < 0.05$). At PS milkings, MY1 and MY2 were higher at 52 and 60 than at 42 kPa ($P < 0.05$). At nPS milkings, MY1 did not differ among vacuum levels, whereas MY2 was higher at 60 than 42 kPa ($P < 0.05$). Bimodalities occurred at 17% in PS and at 91% in nPS milkings. In PS milkings, most bimodalities occurred at 60 kPa (31%) and less frequently at 42 and 52 kPa (12% and 6%, respectively). The highest frequency of bimodalities was observed in nPS milkings at 52 and 60 kPa (93% each), and slightly less at 42 kPa (87%). The dI was longer at PS than nPS milkings at all vacuum levels ($P < 0.05$) but did not differ among vacuum levels. The dP did not differ between PS and nPS milkings at all vacuum levels and was shorter at 60 than 42 kPa at both PS and nPS milkings ($P < 0.05$). The tPFR was longer at PS than nPS milkings, whereas vacuum levels did not affect tPFR ($P < 0.05$). The PFR did not differ between PS and nPS milkings but increased considerably with vacuum levels ($P < 0.05$). The duration of decline neither differed between PS and nPS milkings nor among vacuum levels. The AMF was higher at PS than nPS milkings at all vacuum levels ($P < 0.05$). The AMF was higher at 52 and 60 than at 42 kPa at PS milkings ($P < 0.05$) and tended to be higher at 60 than 42 kPa at nPS milkings ($P = 0.055$). The MOT was shorter at PS than nPS milkings

Table 1. Milking characteristics (means + SEM) at different vacuum levels with (PS) or without (nPS) prestimulation

Milking variable	Treatment ¹					
	PS 42	nPS 42	PS 52	nPS 52	PS 60	nPS 60
Total milk yield (kg)	12.80 ± 1.04	12.70 ± 0.70	12.56 ± 0.99	12.51 ± 0.95	12.46 ± 0.96	11.95 ± 0.91
Milk yield reached in 1 min (kg)	2.92 ± 0.27 ^{a,B}	1.68 ± 0.21 ^b	3.64 ± 0.30 ^{a,A}	1.89 ± 0.26 ^b	3.83 ± 0.34 ^{a,A}	1.83 ± 0.25 ^b
Milk yield reached in 2 min (kg)	6.69 ± 0.47 ^{a,B}	4.28 ± 0.48 ^{b,B}	8.00 ± 0.57 ^{a,A}	4.94 ± 0.58 ^{b,AB}	8.66 ± 0.61 ^{a,A}	5.52 ± 0.71 ^{b,A}
Duration of incline (min)	0.65 ± 0.09 ^b	1.62 ± 0.13 ^a	0.69 ± 0.08 ^b	1.82 ± 0.10 ^a	0.82 ± 0.08 ^b	1.61 ± 0.15 ^a
Duration of plateau (min)	1.69 ± 0.21 ^A	1.40 ± 0.26 ^A	1.22 ± 0.17 ^{A,B}	0.86 ± 0.10 ^{A,B}	0.83 ± 0.11 ^B	0.56 ± 0.10 ^B
Time until peak flow rate (min)	1.79 ± 0.20 ^b	2.72 ± 0.15 ^a	1.59 ± 0.15 ^b	2.44 ± 0.11 ^a	1.46 ± 0.13 ^b	2.27 ± 0.11 ^a
Peak flow rate (kg/min)	3.99 ± 0.23 ^C	3.61 ± 0.26 ^C	4.80 ± 0.30 ^B	4.52 ± 0.27 ^B	5.28 ± 0.29 ^A	5.14 ± 0.27 ^A
Duration of decline (min)	2.70 ± 0.29	3.09 ± 0.41	2.26 ± 0.24	2.33 ± 0.19	2.58 ± 0.26	2.46 ± 0.23
Average milk flow (kg/min)	2.51 ± 0.19 ^{a,B}	2.12 ± 0.17 ^{b,Z}	2.98 ± 0.23 ^{a,A}	2.42 ± 0.18 ^{b,YZ}	2.93 ± 0.23 ^{a,A}	2.52 ± 0.22 ^{b,Y}
Machine-on time (min)	5.26 ± 0.38 ^{b,A}	6.32 ± 0.37 ^{a,A}	4.39 ± 0.34 ^{b,B}	5.23 ± 0.22 ^{a,B}	4.41 ± 0.29 ^B	4.85 ± 0.21 ^B

^{a,b}Means with different lowercase letters differ significantly ($P < 0.05$) between PS or nPS within the same vacuum level.

^{A-C}Means with different uppercase letters differ significantly ($P < 0.05$) between the vacuum level within PS or nPS.

^{Y,Z}Means with different uppercase letters tend to differ ($P = 0.05-0.1$) between the vacuum level within PS or nPS.

¹Number designations indicate different vacuum levels: 42, 52, or 60 kPa.

at 42 and 52 kPa ($P < 0.05$), but not at 60 kPa. At both PS and nPS milkings, the MOT was shorter at 52 and 60 than at 42 kPa ($P < 0.05$).

The claw vacuum at PFR was 33.8 ± 0.4 kPa at PS and 34.3 ± 0.4 kPa at nPS milkings at system vacuum of 42 kPa. At system vacuum of 52 kPa, the claw vacuum was 40.0 ± 1.0 and 41.4 ± 0.6 kPa at PS and nPS milkings, respectively. At the highest system vacuum of 60 kPa, the vacuum at PFR was 46.9 ± 0.6 and 47.3 ± 0.6 kPa at PS and nPS milkings, respectively. In bimodal milk flow curves, the claw vacuum increased transiently after a first decline after attachment and decreased again after alveolar milk ejection and second increase of milk flow (Figure 1).

Ultrasound Imaging of One Gland Cistern

The maximal size of the area of the gland cistern did not differ between PS and nPS milkings and among vacuum levels. The dAI (Table 2) was longer at nPS than PS milkings ($P < 0.05$) but did not differ among vacuum levels. The dAP was longer at PS than nPS milkings, but significantly ($P < 0.05$) only at 52 kPa. At PS milkings, the dAP was shorter at 52 and 60 kPa than at 42 kPa ($P < 0.05$). At nPS milkings, the dAP was shorter at 60 than 42 kPa and tended to be shorter at 52 than at 42 kPa ($P = 0.086$). The tEAP was longer at nPS than PS milkings at all vacuum levels ($P < 0.05$). At nPS milkings, tEAP was shorter at 60 kPa than at 42 kPa ($P < 0.05$) and tended to be shorter at 52 than 42 kPa ($P = 0.069$). At PS milkings, the tEAP did not differ among vacuum levels. The dSAD did not differ between PS and nPS milkings. At PS milkings, the dSAD was longer at 42 kPa than at 52 and 60 kPa ($P < 0.05$). The dFAD did not differ between PS and nPS milkings. At both PS and nPS milkings, the dFAD

was longer at 42 kPa than at 60 kPa ($P < 0.05$) and tended to be longer at 52 kPa than at 42 kPa ($P = 0.087$ and $P = 0.091$, respectively). The EAD was earlier at PS than nPS milkings ($P < 0.05$) at all vacuum levels. In PS milkings, the EAD was reached earlier at 60 kPa than at 52 kPa ($P < 0.05$) and earlier at 52 kPa than at 42 kPa ($P < 0.05$). In nPS milkings, the EAD was reached earlier at 52 kPa than at 42 kPa ($P < 0.05$) and tended to be reached earlier at 60 kPa than at 52 kPa ($P = 0.057$). Figures 2 and 3 each show a representative PS and nPS milking with corresponding ultrasound images.

DISCUSSION

In the present study, we investigated milking characteristics depending on vacuum levels up to 60 kPa and the quarter cisternal size changes throughout PS or nPS milkings. We tested the hypothesis that a high vacuum increases the milking efficiency and enables faster milk removal as long as the continuous milk ejection can maintain adequate cisternal filling with milk.

Neither vacuum levels nor prestimulation affected the TMY in our study. In agreement with previous studies, this confirms that udder emptying was complete even if cisternal filling was transiently reduced due to delayed milk ejection at nPS milkings (Besier and Bruckmaier, 2016; Neuheuser et al., 2018; Rasmussen and Madsen, 2000). On the other hand, several studies found a decreased milk yield at nPS milkings and delayed milk ejection (Rasmussen et al., 1992; Erskine et al., 2019). Differences may be based on the milking routine, milking machine settings, or genetic differences related to the anatomical structure of the mammary tissue and milk duct system. Nevertheless, a high vacuum level and prestimulation led to a higher AMF due to a

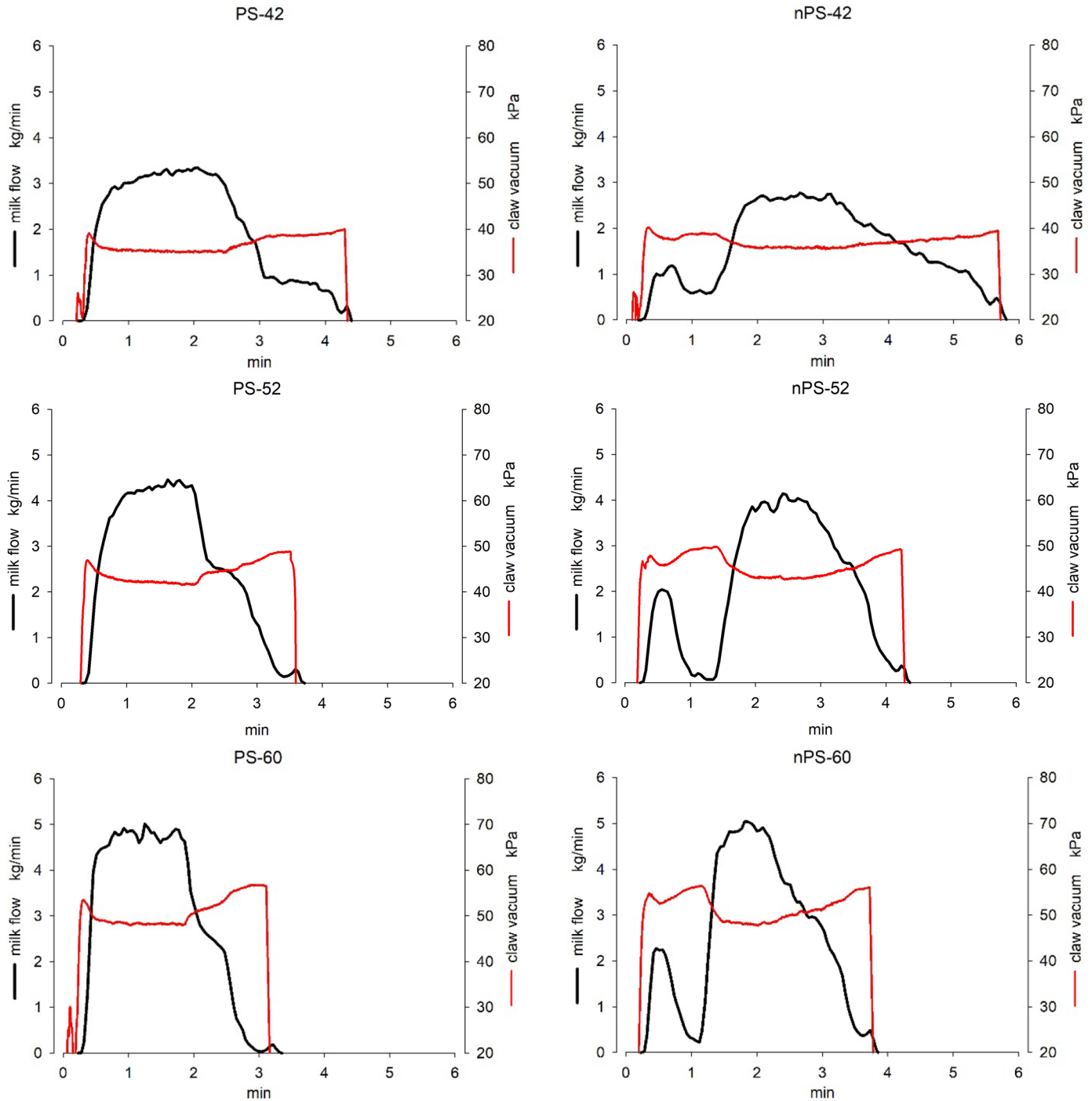


Figure 1. Milk flow and vacuum curves of one representative cow. Milkings were conducted with or without prestimulation (PS = with prestimulation, nPS = without prestimulation) and at different vacuum levels (42, 52, and 60 kPa).

shorter MOT, whereas TMY was unaffected (Weiss and Bruckmaier, 2005).

Milk flow started immediately after cluster attachment in all treatments. As expected, due to the delayed alveolar milk ejection at nPS milkings, milk flow was interrupted after removal of cisternal milk, which re-

sulted in bimodal milk flow patterns (Bruckmaier and Blum, 1996; Sandrucci et al., 2007). The percentage of bimodal milk flow curves was considerably higher in nPS compared with PS milkings, which indicates transient overmilking due to delayed cisternal refill already at the start of milking. This effect is well known,

Table 2. Gland cistern area of 1 front quarter during the course of milking (means + SEM)

Item	Treatment ¹					
	PS 42	nPS 42	PS 52	nPS 52	PS 60	nPS 60
Maximum area (cm ²)	17.7 ± 2.5	16.3 ± 2.1	16.4 ± 2.0	16.5 ± 2.0	17.3 ± 2.0	15.5 ± 1.9
Duration of area increase (min)	0.47 ± 0.13 ^b	1.92 ± 0.12 ^a	0.55 ± 0.12 ^b	1.85 ± 0.15 ^a	0.73 ± 0.17 ^b	1.82 ± 0.12 ^a
Duration of area plateau (min)	1.48 ± 0.25 ^A	1.32 ± 0.23 ^{A,Y}	1.43 ± 0.18 ^{a,A}	0.85 ± 0.08 ^{a,AB,ZY}	1.03 ± 0.13 ^B	0.87 ± 0.15 ^B
Time until end of area plateau (min)	2.02 ± 0.23 ^b	3.23 ± 0.25 ^{a,A,Y}	1.98 ± 0.23 ^b	2.77 ± 0.18 ^{a,AB,Z}	1.77 ± 0.18 ^b	2.7 ± 0.23 ^{a,B}
Duration of slow area decrease (min)	0.77 ± 0.13 ^A	0.57 ± 0.12	0.40 ± 0.08 ^B	0.55 ± 0.10	0.32 ± 0.05 ^B	0.38 ± 0.08
Duration of fast area decrease (min)	0.78 ± 0.15 ^{A,Y}	0.77 ± 0.13 ^{A,Y}	0.58 ± 0.10 ^{AB,Z}	0.53 ± 0.07 ^{AB,Z}	0.47 ± 0.03 ^B	0.43 ± 0.07 ^B
End of area decrease (min)	3.37 ± 0.30 ^{b,A}	4.42 ± 0.33 ^{a,A}	2.87 ± 0.22 ^{b,B}	3.70 ± 0.25 ^{a,B,Y}	2.35 ± 0.15 ^{b,C}	3.13 ± 0.13 ^{a,B,ZZ}

^{a,b}Means with different lowercase letters differ significantly ($P < 0.05$) between PS or nPS within the same vacuum level.

^{A-C}Means with different uppercase letters differ significantly ($P < 0.05$) between the vacuum level within PS or nPS.

^{Y,Z}Means with different uppercase letters tend to differ ($P = 0.05-0.1$) between the vacuum level within PS or nPS.

¹PS = with prestimulation; nPS = no prestimulation. Number designations indicate different vacuum levels: 42, 52, or 60 kPa.

which is due to the delayed induction of oxytocin release and milk ejection in nPS milkings (Bruckmaier and Blum, 1996). However, in both nPS and PS milkings, the percentage of bimodal milk flow curves was higher at high than low vacuum. As expected, the cisternal emptying at the start of milking was so fast that even an incomplete milk ejection in PS milkings caused bimodality and, hence, transient overmilking. An important consequence of overmilking already at the start of milking is the maintained high vacuum level instead of the expected vacuum drop caused by milk flow. Thus, if milking is performed at very high vacuum, a milk flow controlled vacuum reduction during phases of low milk flow or even overmilking are to be recommended both at the start and end of milking to prevent a preterm climbing of the teat cups as well as an increased mechanical load on the teat tissue (Stauffer et al., 2020). At nPS milkings, the milk yield removed during the first minute of milking represents the cisternal milk that is available before milk ejection, whereas the second minute of milking is accompanied by the cisternal refill through milk ejection (Wieland et al., 2020). At PS milkings, part of the alveolar fraction may be already available during the first minute of milking. Therefore, the milk yield removed during the first minute of milking was lower in nPS compared with PS milkings. As expected, the transient emptying of the cistern and subsequent refill at bimodal milk flow was accompanied by a decline and successive increase of the cisternal area recorded by ultrasound during milk ejection. The cisternal enlargement of the gland cistern during milk ejection recorded by ultrasound imaging has been previously observed in response to oxytocin injection (Bruckmaier and Blum, 1992).

As shown in earlier studies, the delayed milk ejection at nPS compared with PS milkings resulted in a longer dI as well as a longer tPFR (Tančin et al., 2007). Although occurring later, the PFR was not affected by

prestimulation in PS milkings. As observed in earlier studies, the claw vacuum dropped depending on the level of milk flow at all used system vacuum settings (Ambord and Bruckmaier, 2010; Besier and Bruckmaier, 2016; Wieland et al., 2021). In bimodal milk flow patterns, the vacuum drop disappeared transiently and reached almost system vacuum levels until the occurrence of milk ejection and second increase of milk flow. This effect was confirmed by the simultaneously longer dAI in the recorded front quarter in nPS than PS.

The vacuum level affected neither the dI of milk flow nor the dAI and maximum size of cisternal area in the recorded front quarter. However, the MY1 and MY2 was higher at 52 and 60 kPa compared with the 42 kPa vacuum. This finding confirms that during the early phase of milking, the milk ejection rate has the potential to fill the cisternal cavities completely within a similar time at all 3 vacuum levels despite faster milk removal at high vacuum. This phenomenon demonstrated that the myoepithelial contraction in the mammary gland builds up an elastic pressure on the alveoli and milk ducts, which enables a continuous filling of the cistern during milk removal even if performed at a very high vacuum level (Bruckmaier et al., 1994). Continuous ultrasound imaging showed that the cisternal size did not decrease throughout milk flow plateau, also demonstrating that the maximum cisternal fill was independent of the applied vacuum level. In agreement with previous studies (Besier and Bruckmaier, 2016), the higher PFR at similar tPFR at milking with high vacuum confirmed that the cistern is similarly filled through milk ejection at all studied vacua.

The higher PFR combined with a shorter dP at the highest tested vacuum level showed a higher milking efficiency at higher vacuum levels but only if abundant milk was available through milk ejection. The shorter dP at high vacuum demonstrates that the milk ejection rate was declining at least in individual quarters

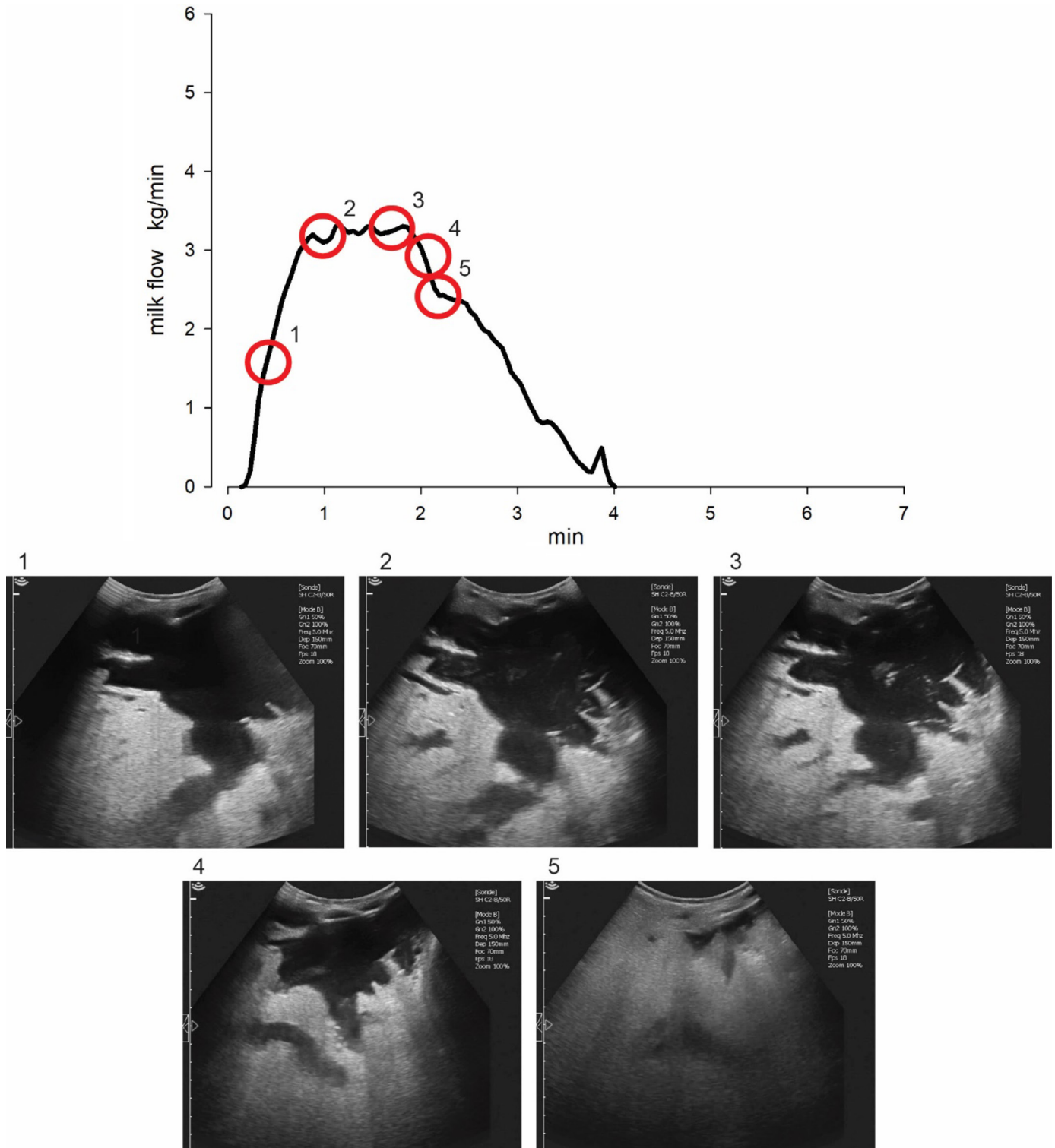


Figure 2. Milk flow curve and corresponding ultrasound images of the cistern of one front quarter of one representative cow. The upper cistern filled with milk appears as a dark area (anechogenic), and the glandular parenchyma as a gray-white area (echogenic). Milking was conducted with prestimulation at a vacuum level of 42 kPa. The red circles show the point in time of the image in the milk flow curve. Due to prestimulation, the cistern was already well filled during the incline phase and stayed at a similar size during the entire plateau phase. During the decline phase, the cistern got smaller until it disappeared when milk flow from the respective quarter stopped.

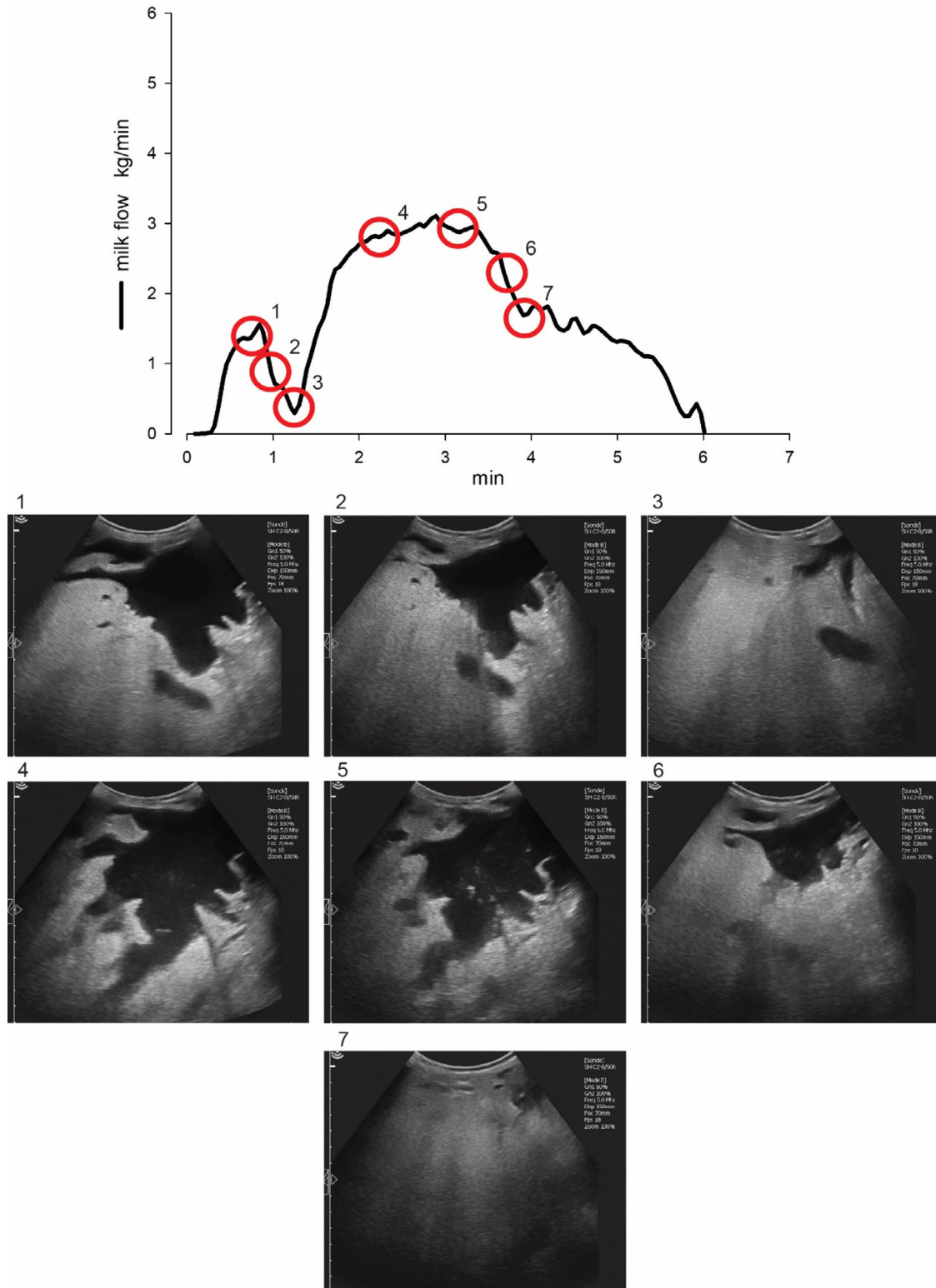


Figure 3. Milk flow curve and corresponding ultrasound images of the cistern of one front quarter of one representative cow. The upper cistern filled with milk appears as a dark area (anechogenic), and the glandular parenchyma as a gray-white area (echogenic). Milking was conducted without prestimulation at a vacuum level of 42 kPa. The red circles show the point in time of the image in the milk flow curve. During the first peak of milk flow the cistern stayed smaller than after in plateau phase. Due to delayed milk ejection, the cistern disappeared before milk ejection occurred and incline phase started. During plateau phase, the cisternal size stayed almost unchanged until it got smaller during decline phase and disappeared when the milk flow from the respective quarter stopped.

before quarter milk flow ceased completely. Because of the faster milk removal, the dP as well as the dAP in the front quarter recorded by ultrasound was shorter at a high than at a low vacuum. The higher the milk flow the earlier the milk ejection rate could no longer keep up with the milk extraction rate by the milking machine. Interestingly, at a 52 kPa vacuum the shortening of the dAP occurred already at nPS but not at PS milkings. This finding indicates that an early induction of milk ejection by prestimulation provides a higher portion of milk in the cistern to allow a longer plateau phase even at a relatively high vacuum level. Regardless of whether prestimulation was performed or not, the cisternal area reached the same maximum size. The tEAP was longer in nPS compared with PS, similarly at all investigated vacuum levels. This difference was obviously caused by the delayed milk ejection in nPS compared with PS, which was represented by the longer dAI in nPS than PS milkings at all vacuum levels.

Despite the differences in the dP, the duration of decline did not significantly differ among PS or nPS milkings as well as at different vacuum settings. The lack of differences in contrast to the results of the ultrasound measurements is likely due to the influence of the 4 quarters resulting in one milk flow curve. The cisternal areas of 1 front quarter showed a slow decrease after the plateau phase, which indicates that the milk flow was still maintained in the respective quarter, whereas the milk ejection rate could no longer keep up with the milk extraction. This finding is indicated by a shorter dSAD at high than at low vacuum, but was significantly visible only at PS milkings. Also, the following shorter dFAD at high than low vacuum demonstrates the faster emptying of the cistern after milk ejection has almost ceased.

CONCLUSIONS

Overall, several milking characteristics appeared to reflect a higher milking efficiency with increasing vacuum up to 60 kPa. This was mainly true during early milking and until PFR (i.e., as long as the alveolar milk ejection rate was similar or higher than the milk extraction rate). However, both AMF and MOT demonstrated that an increase of the vacuum beyond 52 kPa did not necessarily result in higher milking performance throughout the entire milking process. It may be speculated that vacuum levels up to 60 kPa can be applied until the plateau phase of milk flow, also represented by a sufficient cisternal fill through milk ejection, related to the intensity of milk extraction (i.e., a balanced intensity of milk removal which is continuously adjusted to the milk ejection as soon as the milk ejection rate is slowing down). Thus, a controlled reduction of the

vacuum level in low milk flow phase at the start and the end of milking prevents overmilking and increased teat tissue damage in particular if high vacuum settings are used to optimize milking performance.

ACKNOWLEDGMENTS

This research was supported by DeLaval (Tumba, Sweden). We thank Dominik Fritsche (doctoral student, Veterinary Physiology, University of Bern) for his helping hands during the experiments, as well as the team of the Swiss Federal Research Station Agroscope in Posieux, especially Lukas Eggerschwiler and the animal keepers, for organization and support during the experiments. The authors have not stated any other conflicts of interest.

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