The objectives of this study were to assess the effect of 2 different premilking stimulation regimens, with and without a latency period between tactile stimulation and the attachment of the milking unit, on the teat tissue condition and milking performance of dairy cows. In a randomized controlled crossover study, 145 Holstein cows milked 3 times daily were assigned to treatment (TRT) or control (CON) groups. Premilking udder preparation for the TRT group consisted of the application of a latency period resulting in a preparation lag time of 90 s. The only difference in the premilking udder preparation of the CON group was the absence of latency period; the milking unit was attached immediately after completion of the tactile stimulation. The average duration of total tactile stimulation in TRT and CON group was 8 ± 2 and 9 ± 2 s, respectively. The study lasted for 14 d and was split into 2 periods, each consisting of a 2-d adjustment period followed by 5 d of data collection. We assessed machine milking-induced short-term changes to the teat tissue by palpation and visual inspection postmilking. Electronic on-farm milk meters were used to assess milking characteristics (milk yield [kg/milking session], machine-on time [s], 2-min milk yield [kg], and duration of low milk flow rate [s]). Generalized linear mixed models were used to analyze the effect of treatment on the outcome variables. The odds of machine milking-induced short-term changes to the teat tissue by palpation and visual inspection postmilking. Electronic on-farm milk meters were used to assess milking characteristics (milk yield [kg/milking session], machine-on time [s], 2-min milk yield [kg], and duration of low milk flow rate [s]). Generalized linear mixed models were used to analyze the effect of treatment on the outcome variables. The odds of machine milking-induced short-term changes to the teat tissue were lower for cows that received a 90-s preparation lag time (TRT cows) compared with cows in the CON group (odds ratio [95% confidence interval; 95% CI] = 0.13 [0.08–0.20]). The least squares means (95% CI) values of cows in the TRT and CON groups were 15.4 (14.9–15.9) and 15.3 (14.8–15.8) kg, respectively, for milk yield, and 246 (239–253) and 253 (247–260) s for machine-on time. The 2-min milk yield was higher for the TRT compared with CON group cows at all the parity levels. The 2-min milk yields of animals in lactation 1, 2, and ≥3 were 5.7, 5.7, and 6.5 kg, respectively, in the TRT group and 4.6, 5.0, and 5.9 kg in the CON group. The TRT cows spent less time in low milk flow rate compared with CON cows at all parity levels. The durations of low milk flow rate of cows in lactation 1, 2, and ≥3 in the TRT group were 19, 17 and 13 s, respectively, and those in the CON group were 31, 22, and 15 s. In this study, cows that received a latency period, and thus were subjected to a 90-s preparation lag time had lower odds of exhibiting short-term changes to the teat tissue after machine milking, shorter machine-on time, higher 2-min milk yields, and lower durations of low milk flow rates. We conclude that consideration of latency period leading to a 90-s preparation lag time in the premilking stimulation regimen facilitated cows’ milk-ejection reflex. This latency period can alleviate the adverse effects of vacuum-induced forces on teat tissue during machine milking, improve udder health, and promote animal well-being. Key words: milking flow rate, milking routine, oxytocin, premilking udder preparation, latency period

INTRODUCTION

Cow milking regimens are designed to facilitate a gentle, quick, and complete milk harvest. In addition, monitoring mammary health is essential to ensure a high-quality product and animal well-being. Current recommendations for premilking udder preparation include teat sanitation (i.e., premilking teat disinfection, cleaning, and drying of teats) and premilking stimulation (NMC, 2013). Premilking stimulation involves tactile stimulation and milking-unit attachment delay (i.e., the preparation lag time [the period from the first tactile stimulus to the attachment of milking unit] or latency period [the period after completion of
tactile stimulation(s) and before milking-unit attachment). Premilking stimulation is helpful in the establishment of the milk-ejection reflex (Bruckmaier and Blum, 1996), which facilitates gentle, quick, and complete milk harvest particularly the alveolar milk fraction, which can comprise up to 80% of the udder’s milk volume (Pfeilsticker et al., 1996; Bruckmaier and Blum, 1998). A mechanical prestimulation of 90 s was found optimum for achieving immediate and continuous milk flow at the start of milking in udder containing small amount of milk, whereas well-filled udders require only 20 s of prestimulation (Weiss and Bruckmaier, 2005). A preparation lag time of 90 s coupled with forestripping was reported to enhance the milking performance in terms of milk flow rate, 2-min milk yield (2MIN), and machine-on time (MOT) in late-lactation cows with a thrice daily milking schedule (Watters et al., 2012).

From the perspective of practical milking management, a short latency period between udder preparation and the start of milking is advantageous because several animals can be prepared in a row before the clusters are attached. A short premilking stimulation of 15 s followed by a latency period of up to 45 s has been shown to be a suitable alternative to continuous stimulation for milk ejection (Kaskous and Bruckmaier, 2011). Vetter et al. (2014) also reported a similar milking performance when a tactile stimulation of 15 s was coupled with 30 s latency period as compared with 45 s of continuous stimulation. However, a latency period of 2 min or more is not recommended, as the blood oxytocin concentration rapidly declines in the absence of tactile stimulation (Bruckmaier, 2013). This leads to decreased intracister nal pressure along with slow and incomplete udder emptying (Mayer et al., 1984; Bruckmaier and Hilger, 2001). A tactile teat stimulation of 10 to 20 s and a lag time of 60 to 90 s from the beginning of stimulation to the milking-unit attachment are suggested to be ideal for achieving constant letdown and efficient milk removal (Reneau and Chastain, 1995). Consideration of a 90-s preparation lag time has been recommended previously (Wieland and Nydam, 2017) and could be the optimum for evaluating the beneficial effect of a latency period in these scenarios involving cows of all lactation stages with a thrice daily milking schedule.

Recent studies have shown the beneficial effects of preparation lag time on the elicitation of the milk-ejection reflex and milking performance (Watters et al., 2012, 2015). Despite these findings, the adoption of a milking routine that includes a sufficiently long preparation lag time has been implemented slowly, likely due to the increase in herd size and a subsequent increase in reliance on hired labor (Von Keyserlingk et al., 2013; Adcock et al., 2015), which demands greater focus on increasing parlor throughput leading to reduced stimulation time and latency period (Moore-Foster et al., 2019). Sandrucci et al. (2007) reported that as farm size increases, less time is spent on teat stimulation, thus reducing the overall time for udder preparation. Moore-Foster et al. (2019) reported that the total stimulation time was reduced by half in larger herds (herds with ≥300 cows) compared with that in smaller herds. Previous work from our own group also indicated that the preparation lag time applied in large dairy operations is as short as 57 s (Wieland et al., 2021).

However, improper preparation lag time may aggravate the effect of machine milking on teat tissue. In a recent observational study, Wieland et al. (2019) reported that a shorter preparation lag time was associated with increased odds of machine milking-induced short-term teat tissue changes (STC) such as discoloration of teat skin color, swelling at the teat base, firmness of the teat end, and openness of the teat orifice. The STC can lead to increased teat canal colonization (Zeconi et al., 1992), a higher risk of new IMI (Zeconi et al., 1996), and an increased SCC (Zwertvaegher et al., 2013). In addition, STC have been thought to reduce animal well-being (Hillerton et al., 2002). The implementation of a milking regimen that includes a sufficiently long preparation lag time may mitigate these consequences. However, to the best of our knowledge, controlled studies investigating the cause-effect relationship between preparation lag time and STC in high-producing Holstein dairy cows milked 3 times per day are lacking.

Therefore, the primary objective of this study was to investigate the effect of preparation lag time on STC. We hypothesized that the application of a latency period resulting in a 90-s preparation lag time along with tactile stimulation (forestripping and wiping of teats) would reduce the adverse effects of machine milking on teat tissue condition through improved elicitation of the milk-ejection reflex. Our secondary objective was to study the effect of preparation lag time on milking performance. We hypothesized that the application of a latency period that leads to a 90-s preparation lag time would increase the amount of milk harvested, enhance milk flow rates, and decrease the machine-on time.

MATERIALS AND METHODS

This randomized field trial was conducted at the Teaching Dairy Barn of the College of Veterinary Medicine, Cornell University (Ithaca, NY) from June 24 to July 7, 2022. The study protocol was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (protocol no. 2021–0105).
Animals and Housing

The lactating herd consisted of approximately 160 Holstein cows. Cows were housed year-round in 2 freestall pens with sand bedding and were fed a TMR formulated according to NRC requirements (NRC, 2001). The herd key performance indicators were average milk production (13,860 kg), mean test-day SCC (228,000 cells/mL), monthly clinical mastitis incidence (1.3%), and 21-d pregnancy rate (27.0%).

Milking System

Cows were milked 3 times per day at 0400, 1100, and 1900 h in a double-10 parallel milking parlor (P2100, DeLaval International AB, Tumba, Sweden). The vacuum pump (7.5 kW) was regulated by a variable frequency drive and set to supply a receiver operator vacuum of 45 kPa. The milking unit was composed of the cluster MC70 (weight: 2.1 kg; DeLaval International AB) and a milking liner with a square barrel shape (ProSquare DPX2, IBA, Millbury, MA). The pulsators (Delatron, DeLaval International AB) were set to a pulsation rate of 60 cycles/min, a ratio of 70:30, and a side-to-side alternating pulsation. The automatic cluster removers were set to remove the units at a milk flow of 1.4 kg/min with a 0-s delay and a vacuum decay time of 2.3 s. The milk sweep was initiated 1.5 s after unit retraction and lasted for 4 s. The milk line was installed 75 cm below the cow standing level. All milking system settings were verified and assessed by the investigators according to the guidelines outlined by the National Mastitis Council (NMC, 2012) before the start of the study.

Treatment Allocation

Lactating cows were considered for enrollment if they were free of clinical mastitis for at least 2 wk and had no udder abnormalities such as nonlactating quarters or teat injuries. Eligible cows were randomly assigned to treatment (TRT) and control (CON) groups stratified by parity, stage of lactation, and average daily milk yield (MY) during the previous week using a random number generator (Urbaniak and Plous, 2013). The cows of CON group were tagged with leg bands to differentiate them from the TRT group.

The milking routine was performed by 2 operators per milking session. During sessions 1 and 2, the operators were a farm employee and 3 trained investigators. Milking session 3 was operated by students at the Cornell University College of Veterinary Medicine (Ithaca, NY). Premilking udder preparation for the TRT group was performed with sets of 5 cows in 5 steps resulting in 3 visits. Visit 1 included step 1, predipping with 1% iodine (Multi Dose MD; DeLaval International AB) using a teat dip applicator cup. Visit 2 included step 2, sequential forestripping of 2 streams of milk per quarter; step 3, wiping (i.e., drying and cleaning) of teats with a clean cloth towel; and step 4, a latency period. Visit 3 included step 5, attachment of the milking unit with a preparation lag time of 90 s. To facilitate the preparation lag time, we installed a timer (99M/59S Timer, Traceable Products, Webster, TX) at each stall and started the timer at the time of the first forestrip. The 90-s preparation lag time was considered to meet the industry recommendations in our region considering the herd having cows of all lactation stages and a 3 times per day milking schedule (Watters et al., 2012; Wieland and Nydam, 2017). Premilking udder preparation for the CON group was identical except that step 4 was skipped, with the milking unit attached immediately after the wiping step (Figure 1). The duration of forestripping, wiping, and preparation lag time was assessed from a haphazard sample of 24 and 13 milking observations from the TRT and CON groups, respectively. The average (mean ± SD, median [range]) durations for cows in the TRT and CON groups were as follows: forestripping, 4 ± 1, 4 (2–7) and 5 ± 1, 5 (3–8) s; wiping, 4 ± 1, 4 (2–7) and 5 ± 2, 4 (2–8) s; preparation lag time, 88 ± 5, 87 (73–99) and 14 ± 3, 13 (9–19) s. The average (mean ± SD, median [range]) durations of tactile stimulation were 8 ± 2, 8 (4–13) s in the TRT group and 9 ± 2, 9 (6–13) s in the CON group. After milking-unit detachment, all teats were dipped in 1% iodine (Multi Dose MD; DeLaval International AB) using the same method as for predipping. To increase the sample size, this trial was designed as a crossover study. Thus, the first treatment period was 7 d long and consisted of a 2 d of adjustment period followed by 5 d of data collection. On d 8 of the study, cows were switched to the other group and followed for another 7 d (2 d of adjustment time, 5 d of data collection).

Sample Size Considerations

All eligible milking cows in the herd (n = 145) were enrolled. Using the available sample size, we calculated the resulting power. This calculation was based on the primary outcome, the effect of treatment on the occurrence of STC. In a previous study (Wieland et al., 2020), we documented that cows receiving a similar premilking stimulation regimen as the TRT group had 49.7% of cow milking observations with an STC. We assumed that cows not subjected to a latency period would experience STC in at least 70% of cow milking observations. Using an α level of 0.05 and an equal
Sample distribution, the available sample size of 290 cows resulted in a power of 91% for the detection of a 20-percentage point difference (50% vs. 70%) between the 2 groups. The available sample size was also sufficient to detect a MY difference of 1 kg/milking session between the 2 groups at an α level of 0.05 with a power of 0.86. This calculation was based on a reported standard deviation of 3.9 kg for MY (Wieland et al., 2020), presumed correlation between measurements within a cow of 0.5, a total of 30 milking observations, and a repeated-measures ANOVA with an equal sample proportion; the sample size was calculated using G*Power version 3.1.9.2 (Faul et al., 2007).

Data Acquisition

Cow Characteristics. Cow characteristics such as parity, DIM, and 305-d mature-equivalent milk production were obtained from the dairy management software program (DairyComp 305, Valley Agricultural Software, Tulare, CA).

Milking Characteristics. Milking characteristics (MY [kg], first 15 s milk flow rate [kg/min], 15 to 30 s milk flow rate [kg/min], 30 to 60 s milk flow rate [kg/min], 60 to 120 s milk flow rate [kg/min], average milk flow rate [AMF; kg/min], peak milk flow rate [PMF; kg/min], 2MIN [kg], duration of low milk flow rate [LMF; s], and MOT [s]) were assessed at each milking with electronic on-farm milk meters using near-infrared technology (MM27BC, DeLaval International AB) and recorded using dairy farm management software (DePro, DeLaval International AB). The detailed definitions of various milking characteristics and abbreviations used are listed in Table 1. For subsequent analysis, a new categorical variable (i.e., bimodal milk flow) was created and defined as previously described (Wieland et al., 2020; Wieland and Sipka, 2023a): bimodal milk flow was present if any of the incremental milk flow rates (flow rates during 15–30 s, 30–60 s, or 60–120 s) were lower than any of the previous rates (flow rates during the first 15 s, 15–30 s, or 30–60 s).

Milking Irregularities. The occurrences of a milking liner slip, and a milking unit kick-off were monitored with electronic on-farm milk meters in conjunction with the software program. The threshold limit for milking liner slip was set to 175. The occurrence of milking-unit reattachment was registered via the milking point controller (MPC680, DeLaval International AB) and documented with the software program.

Teat Tissue Condition. The assessment of the postmilking teat tissue condition was performed by one trained investigator during session 2 on d 5, 6, and
Table 1. Definitions of milking characteristics recorded with the electronic on-farm milk meter (MM27BC, DeLaval International AB, Tumba, Sweden)

<table>
<thead>
<tr>
<th>Milking characteristic</th>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield (kg)</td>
<td>MY</td>
<td>Yield of milk recorded from the start of milking(^1) to detachment of milking unit</td>
</tr>
<tr>
<td>First 15 s milk flow rate (kg/min)</td>
<td>15S</td>
<td>Average milk flow rate recorded in the first 15 s after the start of milking(^1)</td>
</tr>
<tr>
<td>15 to 30 s milk flow rate (kg/min)</td>
<td>30S</td>
<td>Average milk flow rate between 15 to 30 s after the start of milking(^1)</td>
</tr>
<tr>
<td>30 to 60 s milk flow rate (kg/min)</td>
<td>60S</td>
<td>Average milk flow rate between 30 to 60 s after the start of milking(^1)</td>
</tr>
<tr>
<td>60 to 120 s milk flow rate (kg/min)</td>
<td>120S</td>
<td>Average milk flow rate between 60 to 120 s after the start of milking(^1)</td>
</tr>
<tr>
<td>Average milk flow rate (kg/min)</td>
<td>AMF</td>
<td>Total milk yield/milking unit-on time</td>
</tr>
<tr>
<td>Peak milk flow rate (kg/min)</td>
<td>PMF</td>
<td>Maximum 60-s average milk flow rate</td>
</tr>
<tr>
<td>2-min milk yield (kg)</td>
<td>2MIN</td>
<td>Milk yield harvested within the first 2 min after the start of milking(^1)</td>
</tr>
<tr>
<td>Duration of low milk flow rate (s)</td>
<td>LMF</td>
<td>Duration (s) with a milk flow rate &lt;1 kg/min</td>
</tr>
<tr>
<td>Machine-on time (s)</td>
<td>MOT</td>
<td>Time recorded from the start of milking(^1) to unit detachment</td>
</tr>
</tbody>
</table>

\(^1\)Start of milking: start of the milking procedure as recorded by the milking point controller induced by pushing the start button.
and MOT (<100 or >800 s) as well as observations from cows that were milked with a bucket and those with a deviation from the assigned treatment protocol. To study the effect of treatment on the milking characteristics MY, MOT, 2MIN, and LMF, 4 separate general linear mixed models were fitted with PROC MIXED. The following steps were consistent for all 4 models. To account for the clustering of milking observations within day, cow, and treatment period, a random effect for cow crossed with day and study period was included. Treatment and order of treatment application were forced into the model as fixed effects. The inclusion of additional independent variables in the model was performed as described above. No collinearity was observed among eligible variables. Confounding was assessed by observing regression coefficient changes. Variables that modified regression coefficients by >20% were considered confounding factors. No confounding was observed. Tukey-Kramer post hoc tests were used to account for multiple comparisons. For all final models, the assumptions of homoscedasticity and normality of residuals were assessed by the inspection of residual plots versus corresponding predicted values and the examination of quantile–quantile residual plots. To satisfy these assumptions, values of the dependent variables MOT and LMF were log-transformed. The resulting least squares means (LSM) estimates were back transformed and are presented as the geometric mean and 95% confidence interval.

**Bimodality and Milking Irregularities.** To determine the effect of treatment on bimodality, milking liner slips, milking unit kick-offs, and milking-unit reattachment, 4 separate generalized linear mixed models with a logit link and a binomial distribution were generated with PROC GLIMMIX. The models were constructed in accordance with the procedure outlined above for the effect of treatment on STC with the inclusion of 3 levels of milking sessions (sessions 1, 2, and 3) as covariates.

**Vacuum Measurements.** Differences between groups in the duration of overmilking, average cyclical vacuum fluctuations, mouthpiece chamber vacuum values during the peak flow period, and mouthpiece chamber vacuum during the first 2 min of milking in the right hind and left front teat cups were assessed with Student’s *t*-tests using PROC TTEST.

**RESULTS**

**Description of the Study Population**

The average (mean ± SD) DIM of the 145 study cows at the day of enrollment was 171 ± 107, ranging from 15 to 426 d. Parity was distributed as follows: 53 (36.5%) were in their first lactation, 51 (35.2%) were in their second lactation, and 41 (28.3%) were in their third or greater lactation. The average (mean ± SD) daily MY during the 1 wk before enrollment was 44.9 ± 9.9 kg and ranged from 13.6 to 73.9 kg. The median SCC before the study was 30,000 cells/mL, ranging from 5,000 to 3,563,000. Table 2 displays the baseline characteristics of both study groups.

**Teat Tissue Condition**

We collected and used data of 796 cow observations in the final analyses. The presence of STC was recorded in
246/390 (63%) and 375/406 (92%) cow observations in the TRT and CON groups, respectively. The final model for the presence or absence of STC is shown in Supplemental Table S1 (https://github.com/matthiaswieland/premilking-stimulation-and-milking-performance; Singh et al., 2024) and illustrated in Figure 2. The order of treatment application had no meaningful effect on STC ($P = 0.79$). The odds of STC were lower for cows in the TRT group than for cows in the CON group (odds ratio [95% CI] = 0.13 [0.08–0.20]). The cows in early lactation had higher odds of STC than mid- and late-lactation cows regardless of treatment. Using cows with >200 DIM as the reference groups, the odds ratios (95% CI) were 1.70 (1.08–2.67) for cows with 1 to 100 DIM and 0.76 (0.49–1.18) for cows with 101 to 200 DIM.

**Milking Characteristics**

A total of 4,310 milking observations were obtained. Screening of data resulted in the removal of 476 (11.0%) observations. Manual milking mode and deviation from the study protocol were documented in 11 (0.3%) and 2 (0.1%) cases, respectively. We included the remaining 3,834 milking observations in the final analyses. The average (mean ± SD [median; range]) MY, MOT, 2MIN, and LMF values during the study period were 15.2 ± 4.5 (14.8; 2.6–35.5) kg, 257 ± 69 (253; 100–649) s, 5.5 ± 2.3 (5.5; 0–13.8) kg, and 29 ± 38 (14; 0–480) s, respectively. Table 3 shows the descriptive statistics of milking characteristics stratified by treatment. The effect of a latency period on milking performance was evaluated based on the outcome variables MY, MOT, 2MIN, and LMF. The results of general linear mixed models for each outcome variable are shown in Supplemental Tables S2, S3, S4, and S5 (https://github.com/matthiaswieland/premilking-stimulation-and-milking-performance; Singh et al., 2024) and summarized in Figure 3. There was no meaningful effect of the order of treatment application on MY ($P = 0.59$), MOT ($P = 0.81$), 2MIN ($P = 0.88$), LMF ($P = 0.30$), or bimodality ($P = 0.56$). For MOT, the likelihood that group differences were due to chance was 7% ($P = 0.07$). We detected no meaningful differences in MY ($P = 0.57$) among groups. The LSM values (95% CI) for cows in the TRT and CON groups were 15.4 (14.9–15.9) and 15.3 (14.8–15.8) kg for MY, respectively, and 246 (239–253) and 253 (247–260) s for MOT. There was an interaction between treatment and parity on 2MIN and LMF. The 2MIN values in animals in first, second, and third or greater lactation were 5.7 (5.3–6.2), 5.7 (5.3–6.2), and 6.5 (6.0–7.0) kg, respectively, for TRT cows, and 4.7 (4.2–5.1), 5.1 (4.6–5.6), and 5.9 (5.4–6.4) kg for CON cows. Tukey-Kramer post hoc tests revealed differences between treatments among cows in first, second, and third or greater lactation ($P < 0.0001$). The LMF values in cows in first, second, and third or greater lactation were 19 (17–22), 17 (15–20), and 13 (11–14) s, respectively, in the TRT group and 31 (27–32), 22 (20–23), and 15 (13–18) s in the CON group. Tukey-Kramer post hoc tests revealed differences between treatments among all 3 parity stages ($P \leq 0.01$). Cows in late lactation had lower 2MIN compared with early and mid-lactation animals ($P \leq 0.04$).

Bimodal milk flow was documented in 1,934/3,834 (50.4%) milking observations. The final model included treatment ($P < 0.0001$), parity ($P < 0.0001$) and the interaction between treatment and parity ($P = 0.002$; Supplemental Table S6; https://github.com/matthiaswieland/premilking-stimulation-and-milking-performance; Singh et al., 2024). The TRT group cows had shown lower odds of bimodality as compared with the CON group at all parity levels. Figure 4 depicts the adjusted probabilities, illustrating the interaction between treatment and parity.
Frequency distributions for milking irregularities (milking liner slips, milking unit kick-offs, and milking-unit reattachment) among milking observations were 455/3,834 (11.9%), 265/3,834 (6.9%), and 244/3,834 (6.4%), respectively. Table 3 shows frequency distributions for milking irregularities stratified by group. The results of the final models for milking liner slips, milking unit kick-offs, and milking-unit reattachment are shown in Supplemental Tables S7, S8, and S9 (https://github.com/matthiaswieland/premilking-stimulation-and-milking-performance; Singh et al., 2024). The order of treatment allocation had no meaningful effect on liner slips ($P = 0.14$), whereas the order of treatment allocation influenced the presence or absence of milking unit kick-offs ($P = 0.049$) and milking-unit reattachment ($P = 0.04$). Cows subjected to a latency period in the beginning followed by no latency in the premilking udder preparation had shown decreased odds of a kick-off (OR [95% CI] = 0.74 [0.77–1.00]) and a reattachment of the milking unit (OR [95% CI] = 0.74 [0.55–0.98]), compared with cows that started with CON and were then switched to TRT. The adjusted probabilities of variables in response to treatment are depicted in Figure 5. There was no effect of treatment on milking liner slips ($P = 0.14$), milking unit kick-offs ($P = 0.34$), or milking-unit reattachment ($P = 0.85$).

### Vacuum Measurements

We obtained vacuum measurements of 48 cow milking observations (TRT, 22; CON, 26). The average (mean ± SD [median; range]) durations of overmilking, average cyclical vacuum fluctuation and mouthpiece chamber vacuum for cows in the TRT and CON groups were 48 ± 32 (44; 10–141) and 54 ± 47 (44; 7–214) s, respectively; 37.4 ± 20.0 (38.0; 32.7–41.7) and 37.1 ± 31.3 (37.8; 26.4–40.4) kPa; and 12.2 ± 7.4 (12.9; 4.3–32.0) and 14.5 ± 5.5 (14.5; 5.3–32.0) kPa. We observed no meaningful differences between groups ($P ≥ 0.11$). The average mouthpiece chamber vacuum values during the first 2 min of milking in the TRT and CON groups were as follows: right hind, 14.1 ± 6.5 (14.1; 4.2–30.0) kPa and 20.0 ± 7.2 (19.5; 5.2–35.3) kPa, respectively, and left front, 9.0 ± 6.7 (7.7; 1.7–25.2) and 15.0 ± 7.7 (15; 2.2–27.9) kPa, respectively, and were lower for animals in the TRT group ($P ≤ 0.007$).
DISCUSSION

Teat Tissue Condition

In this study, we investigated the effect of preparation lag time on STC in high-producing Holstein dairy cows milked 3 times per day. Our data showed that cows that received a preparation lag time of 90 s had lower odds of STC than cows that were subjected to immediate milking-unit attachment after tactile stimulation (i.e., manual forestripping and wiping of teats). Previous research has shown that STC can increase the risk of new IMI (Zeconi et al., 1996) and affect the well-being of dairy cows (Hillerton et al., 2002). Our results are thus of particular importance, as they indicate an efficient measure to improve the udder health and well-being of dairy cows.

We speculate that the decreased odds of STC in cows receiving a preparation lag time of 90 s can be attributed to the following factors. First, TRT cows had a shorter MOT than CON cows. This decreased the duration that teats were subjected to vacuum-induced strain, which can cause STC of the teat tissue (Williams and Mein, 1982). Second, cows in the TRT group

Figure 3. Least squares means from general linear mixed models showing the effect of 2 different premilking stimulation regimens on the milk yield (A), 2-min milk yield (B), milking unit-on time (C), and duration of low milk flow rate (D). B and D: P-values for the effect of treatment between cows of different parity are derived from Tukey-Kramer post hoc tests. Error bars represent 95% CI. For other main effects, see Supplemental Tables S2, S3, S4, and S5 (https://github.com/matthiaswieland/premilking-stimulation-and-milking-performance; Singh et al., 2024). TRT = treatment; CON = control.

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had higher milk flow rates and lower durations of low milk flow rates. Due to the inverse relationship between the milk flow rate and vacuum-induced strain to teat tissue (Bade et al., 2009; Ambord and Bruckmaier, 2010), TRT cows likely experienced a lower magnitude of forces responsible for STC of the teat tissue. This is supported by the vacuum measurement results showing that the mouthpiece chamber vacuum values during the first 2 min of milking were lower in TRT cows. Reinemann et al. (2021) also reported decreased odds of blue teats and teats ringing by 12% and 8%, respectively, with a reduction in MOT by 10 s. Third, cows in the TRT group were given more time to elicit the milk-ejection reflex. This may have resulted in ejection of milk into the gland cisterns and teat cisterns, thereby increasing the intracisternal pressure at the time of milking-unit attachment. Increased cisternal pressure could also increase teat diameter and result in a better seal between the teat and the milking liner barrel. As shown by the vacuum measurements, this led to a lower mouthpiece chamber vacuum, which in turn may have resulted in less STC, as suggested by Penry et al. (2017). The higher odds of STC in early lactation animals are consistent with previous findings from our group (Wieland et al., 2019, 2020) and are likely attributable to the higher MY and longer subsequent MOT in early lactation animals. The longer MOT likely exerted more stress on the teat tissue, resulting in higher odds of STC (Williams and Mein, 1982). In this study, we observed a higher incidence of STC in cows compared with previous studies (Wieland et al., 2019, 2020; Reinemann et al., 2021). This could be partially due to the composite assessment at udder level rather than the evaluation at the teat or quarter level. Another possible

Figure 4. Adjusted probabilities (Adj. Prob.) from generalized linear mixed models showing the effect of 2 different premilking stimulation regimens on bimodality. Points with different letters (a–d) differ at a level of $P < 0.05$ according to Tukey-Kramer post hoc tests. Error bars represent 95% CI. For other main effects, see Supplemental Table S6 (https://github.com/matthiaswieland/premilking-stimulation-and-milking-performance; Singh et al., 2024). TRT = treatment; CON = control.

Figure 5. Adjusted probabilities (Adj. Prob.) from generalized linear mixed models showing the effect of 2 different premilking stimulation regimens on milking liner slips (A), milking unit kick-off (B), and milking-unit reattachment (C). Error bars represent 95% CI. For other main effects, see Supplemental Tables S7, S8, and S9 (https://github.com/matthiaswieland/premilking-stimulation-and-milking-performance; Singh et al., 2024). TRT = treatment; CON = control.
explanation could be the short milking interval between milking sessions 1 and 2 (i.e., 7 h). This may have hampered the teats’ ability to completely recover from STC from the previous milking session (i.e., session 1) leading to an accumulative effect of STC at session 2 when the assessment was performed. The shorter duration of the tactile stimulations (8 and 9 s) in the present study as compared with the recommended duration of 15 s and those applied in previous studies (11 s [Wieland et al., 2019]; 16 s [Wieland et al., 2020]) could have also contributed toward the higher incidence of STC in both group cows.

**Milking Characteristics**

Our second objective was to study the effect of preparation lag time on milking characteristics. We found that cows subjected to a preparation lag time of 90 s had higher 2MIN, lower LMF, shorter MOT, and lower odds of bimodality. In contrast, no meaningful differences in MY were detected between the 2 groups.

The observed differences are most likely due to the additional time available between the wiping step and the attachment of the milking unit for TRT cows. This latency period likely provided more time for the release of oxytocin into circulation, its transportation to the target tissue (i.e., the udder parenchyma), and its binding to the receptors of the myoepithelial cells, resulting in better milk ejection at the time of milking-unit attachment. Adequate milk ejection increased the amount of cisternal milk available for harvesting and thus accelerated the milk harvesting process. In this study population, the attachment of the milking unit immediately after forestripping and wiping did not provide sufficient time to accommodate the cows’ physiological requirement for elicitation of milk-ejection. Our results support current recommendations for adequate milk ejection that teats should be stimulated for at least 15 s (Kaskous and Bruckmaier, 2011; Vetter et al., 2014; Wieland et al., 2020) combined with a preparation lag time of 60 to 120 s (NMC, 2013).

The absence of an effect of different premilking stimulation regimens on MY has been reported previously (Sagi et al., 1980; Merrill et al., 1987; Kaskous and Bruckmaier, 2011; Edwards et al., 2013). However, some studies have reported differences in MY, although these differences were not observed in all groups (Rasmussen et al., 1992; Watters et al., 2015). For example, Rasmussen et al. (1992) conducted 4 experiments with American Holstein, Danish Holstein, and Danish Jersey cows. The treatments consisted of preparation lag times of 0.5, 1.3, and 3 min along with varying durations of teat preparation, which consisted of wiping of teats for 6 or 20 s combined with forestripping of 1 or 5 streams from each teat. They observed that preparation lag time did not affect the MY of Danish or American Holstein cows. Danish Jersey cows showed a lower MY when the preparation lag time was 3 min rather than 0.5 min, whereas the MY in the 1.3-min group was not different from either of the 2 other groups (Rasmussen et al., 1992).

Watters et al. (2015) used 30 Holstein cows to study the effect of manual and mechanical teat stimulation on oxytocin release and milking characteristics. The treatments consisted of (1) immediate attachment of the milking unit, (2) forestripping of 2 streams per teat and drying of teats along with a preparation lag time of 30 s, (3) forestripping and drying of teats along with a 90-s preparation lag time, (4) immediate milking-unit attachment followed by 30 s of mechanical stimulation, and (5) immediate milking-unit attachment with 90 s of mechanical stimulation. The researchers reported higher MY in cows that were subjected to immediate attachment of the milking unit (group 1) and those subjected to immediate milking-unit attachment followed by 30 s of mechanical stimulation (group 4) in comparison with the other 3 groups (Watters et al., 2015).

The absence of a meaningful difference in the MY with different premilking stimulation methods in recent studies could be due to the continuous evolvement in the udder and duct system of cows. That is, we speculate that breeding cows for higher production have modified the udder anatomy of the milk duct system over a period. This altered udder and teat anatomy may facilitate complete drainage of milk irrespectively of the premilking stimulation regimen. This speculation is also supported by recent studies where no differences in the strip yield (residual milk) was reported at the end of milking when different premilking stimulations were applied (Merrill et al., 1987; Rasmussen et al., 1992; Edwards et al., 2013). However, the duration of premilking stimulation seems to influence other milking characteristics such as MOT, 2MIN, and LMF.

The observed differences in MOT between groups in this study are consistent with findings from previous works investigating the effect of different premilking stimulation regimens on milking characteristics (Gorewit and Gassman, 1985; Merrill et al., 1987; Edwards et al., 2013; Watters et al., 2015). Watters et al. (2012) conducted a study to determine the effect of preparation lag time (0, 60, 90, 120, or 240 s) coupled with drying of teats (or drying and forestripping) and the effect of their interaction on milking characteristics among 786 Holstein cows in a commercial herd. They reported that late-lactation cows had shorter MOT when the preparation lag time was 90 s or longer, whereas no differences in the MOT were observed when preparation...
lag times of 0, 60, 90, 120, or 240 s were applied to early and mid-lactation cows (Watters et al., 2012).

A difference of 7 s in MOT can have an effect on the parlor throughput of dairy farms. For example, a farm with a 100-stall rotary parlor and a thrice daily milking schedule with a rotational speed of 490 s/rotation can milk approximately 5,100 cows in 7 h (7 × 60 × 60/490 = 51.4 rotation in 7 h). An increase in the rotational speed by 7 s (483 s/rotation) results in a theoretical throughput of 5,200 cows in 7 h (7 × 60 × 60/483 = 52.2 rotation in 7 h). This could give the farm a provision to accommodate 100 more cows with the existing parlor adding to the dairy’s profitability. A previous study of Reinemann et al. (2021) has also described the effect of decreased MOT on parlor throughput of a 60-unit rotary parlor using bio-economic modeling and showed that reduction in milking duration of individual cows resulted in a shorter total milking time in rotary milking parlors where cows have their milking clusters taken off at the end of their first rotation or when 20% of the cows go on a second rotation.

Studies from Schukken et al. (2005) and Watters et al. (2012) also reported differences in 2MIN among cows that received different premilking stimulation regimens. Watters et al. (2012) reported that a larger fraction of total milk was harvested in the first 2 min of milking when preparation lag times of 90 or 120 s with or without forestripping were applied to early and mid-lactation cows, as well as to late-lactation cows when coupled with forestripping, in comparison with 0 or 60 s of preparation lag time.

As expected, cows that received a 90-s preparation lag time had a shorter LMF. The effect of treatment was modified by parity, with differences among groups decreasing with increasing parity. The application of a 90-s preparation lag time reduced LMF by 12, 5, and 2 s in animals in first, second, and third or greater lactations, respectively. We believe that this is attributable to a larger size of the teat and gland cisterns with increased parity, as reported previously (Mielke, 1969; Knight and Dewhurst, 1994; Pfeilsticker et al., 1996). The larger cistern size in multiparous animals likely resulted in a higher fraction of cisternal milk available for harvest before premilking udder preparation. The larger amount of milk could have facilitated milk harvest at a higher flow rate (i.e., >1 kg/min) and could have taken longer to drain, shortening (or bridging) the gap between the exhaustion of cisternal milk and the availability of the alveolar milk fraction. The observed effect of the interaction between treatment and parity on the dependent variable (presence of bimodality) also supports this theory.

Differences in the odds of bimodality were also observed between groups, as expected. There was an interaction between treatment and parity, with differences between treatment groups greater for animals in their first and second lactation. Our findings are in accordance with those from previous works (Watters et al., 2012, 2015; Fernandes et al., 2023). Fernandes et al. (2023) collected vacuum measurement recordings from 606 cow milking observations at 21 dairy farms and reported that the odds of bimodality decreased with increasing preparation lag time.

We observed higher odds of bimodal milk flow at the cow level with increasing DIM, which agrees with previous studies (Bruckmaier and Hilger, 2001; Weiss and Bruckmaier, 2005; Sandrunci et al., 2007; Samoré et al., 2011). The degree of udder filling decreases after peak production (Bruckmaier and Hilger, 2001) due to a lower milk secretion rate with increasing DIM (Knight and Dewhurst, 1994; Pfeilsticker et al., 1996). Consequently, a stronger and more complete myoepithelial contraction is required to transfer the milk from alveoli to the cistern (Weiss and Bruckmaier, 2005); thus, more time is needed to elicit the milk ejection reflex. Additionally, the transfer of milk from the alveolar compartment to the gland cistern between milkings decreases with the advancement of lactation; therefore, the amount of milk available in the cistern and large ducts is low before milking (Pfeilsticker et al., 1996; Ayadi et al., 2003). Other studies that investigated several combinations of stimulation and preparation lag times reported a decrease in bimodality with increasing preparation lag times (Rasmussen et al., 1992; Kaskous and Bruckmaier, 2011; Watters et al., 2012), with the decrease more evident in later stages of lactation (Kaskous and Bruckmaier, 2011; Watters et al., 2012).

**Milking Irregularities**

We also extended our secondary objective to assess the effect of treatment on milking irregularities, in accordance with a previous study (Wieland et al., 2020). Our results did not show a treatment effect on milking liner slips, milking unit kick-offs, or milking-unit reattachment. The higher risk of liner slips with increasing parity and during early and mid-lactation as compared with late lactation, corresponded to a higher 2MIN (Supplemental Table S4). A similar finding of increased risk of liner slips with increasing parity was reported by Rogers and Spencer (1991). Wieland and Sipka (2023b) reported higher risk of liner slips with increasing 2MIN and decreased risk of liner slips with increasing DIM. This could be due to the flow-dependent vacuum drop affecting the forces acting against the liner slip. Due to the inverse relationship between milk flow and teat-end vacuum (Bade et al., 2009; Ambord and Bruckmaier, 2010), the vacuum drop at the teat level may result in
failure to maintain the frictional force between the teat and the milking liner to hold the milking liner in place. As discussed previously (Spencer and Rogers, 1991; Rasmussen and Madsen, 2000; Wieland and Sipka, 2023b), displacement of the milking liner may break the seal between the teat and milking liner leading to a liner slip. Conversely, the difference in 2MIN between the TRT and CON groups did not produce a marked drop in vacuum to trigger the liner slip.

We observed no differences in kick-offs between the groups as expected by virtue of difference in their premilking stimulation. We attributed this to the fact, that the on-farm milk flow meter only detects kick-offs in extreme scenarios when the cow’s reaction results in a partial or complete detachment of the cluster leading to a substantial air leakage. Although cows often express their discomfort in terms of stepping or kicking during milking (Cerqueira et al., 2017), it does not always result in the partial or complete detachment of the milking unit that is necessary to trigger the air leakage. It is therefore possible that the detection method for kick-offs based on air leakage and unit detachment used in this study, does not reflect the discomfort imposed due to discrepancies in stimulation. A measurement of hind-leg activity during milking as suggested by Raoul et al. (2021) may be a more reliable way to represent the cow discomfort.

**Study Limitations and Future Directions**

Although the study was designed to simulate the current milking practices in modern dairy operations, some limitations must be considered. First, the study spanned only 14 d, and no udder health parameters were considered. A study on preparation lag time encompassing the whole lactation period could provide a broader perspective on the economically viable outcome variables MY, udder health indices, SCC, and clinical mastitis. Second, cows in the CON group were tagged with leg bands for identification. Although the measurements of most of the outcome variables were performed using mechanical means, the observer was not blinded to the treatment during the assessment of STC, which may have resulted in information bias. Third, though we analyzed a small sample from the study population for overmilking and found no difference between the group. There could be possibilities that some STC were attributed due to overmilking during the later stage of milking also, not solely due to lack of premilking stimulation. This might have also increased the overall incidence of STC in both the groups. Forth, we conducted our study on one New York dairy farm with Holstein cows that were milked 3 times per day. Therefore, the external validity may be limited to similar operations in this region. Finally, the parlor efficiency metrics were not assessed for obvious reasons. Future research should therefore investigate and identify opportunities to accommodate the physiological requirements of cows while enhancing parlor efficiency. This could be accomplished through a combination of tactile stimulation and latency periods using inherent systems such as the pulsator system.

**CONCLUSIONS**

Cows that received a latency period between tactile stimulation and the attachment of the milking unit resulting in a 90-s preparation lag time during premilkingudder preparation had lower odds of machine milking-induced STC, shorter milking unit-on time, and spent less time below 1 kg/min milk flow rate to harvest the same amount of milk per milking session. Thus, the application of latency period to achieve a preparation lag time of 90 s coupled with forestripping and wiping of teats in premilkingudder preparation provided more time to accommodate the cows’ physiological requirements for the elicitation of milk-ejection reflex. This alleviated the negative effects of mechanical forces on the teat tissue during machine milking and has the potential to improve udder health and promote animal well-being.

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**ORCIDS**

A. Singh • https://orcid.org/0000-0002-8762-7097
C. M. Geary • https://orcid.org/0000-0002-3027-3980
S. J. Womack • https://orcid.org/0000-0003-4417-817X
M. Dahl • https://orcid.org/0000-0002-3595-1397
M. Wieland • https://orcid.org/0000-0003-0513-1782