Effects of Cleaning Duration and Water Temperature on Oxytocin Release and Milk Removal in an Automatic Milking System

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ABSTRACT

Four different methods of teat preparation during milking in an automatic milking system were studied in 2 experiments on Red Holstein/German Fleckvieh crossbreed cows. Milking routines used were milking: 1) without premilking teat preparation; 2) with one cleaning cycle (58 to 60 s) with cold (13 to 15°C) water; 3) with one cleaning cycle with warm water (30 to 32°C); or 4) with 2 cleaning cycles (122 s) with warm water. In experiment 1, milking characteristics were evaluated and milking routines were randomly assigned to 62 cows during 3 measuring periods of 24 h each. In experiment 2, 10 randomly selected cows were assigned to the same milking routines during 4 d and blood samples for oxytocin (OT) determination were taken during milking in addition to milk flow recording. Milk production, peak flow rate, total, and quarter milk yields showed no differences among treatments. Premilking preparation with cold water compared with warm water showed no differences in OT release, milk yield, peak flow rate, main milking time, average flow rate, or time until main milk flow. Baseline OT concentrations were consistently low. At the start of teat cup attachment without premilking teat preparation OT concentrations remained on the basal level but were elevated in all other treatments. By 30 s from the start of milking, OT concentrations were markedly increased in all treatments and were no longer different between treatments. In conclusion, the teat cleaning device used in the automatic milking system, either with warm or cold water, was suitable to induce milk ejection in cows before the start of milking.

(**Key words:** automatic milking, oxytocin, milk ejection, milking characteristic)

Abbreviation key: AMS = automatic milking system, OT = oxytocin.

INTRODUCTION

Tactile stimulation (manual or mechanical) of the mammary gland causes alveolar milk ejection through a neuro-endocrine reflex arc. In conventional milking, premilking teat preparation is performed to ensure clean teats and complete milk ejection before milking is started. Premilking teat preparation in automatic milking systems (AMS) differs from that in conventional milking. Four different principles of the premilking teat preparation are available in AMS (De Koning et al., 2002). Premilking teat preparation with simultaneous brushing of all 4 teats stimulates oxytocin (OT) release and milk ejection in a multi-box system (Macuhova et al., 2003), as well as sequential teat cleaning by brushes for 32 s in a single stall AMS (Dzidic et al., 2002). Premilking teat preparation with cold water (10°C) compared with warm water (40 or 46°C) showed no differences in milking characteristics during conventional milking (Dodd and Foot, 1947; Frommholdt, 1973). Only in a study of Jersey cows was a prolonged stimulation required if cold water was used (Phillips, 1984). Automatic milking system milking is performed at variable intervals. Commencement of milk ejection is delayed in late lactation or if there had only been a short interval from the previous milking (Bruckmaier and Hilger, 2001). Short milking intervals in AMS increased the milk production rate of high producing cows, whereas milk flow rate was lower in comparison with long milking intervals (Hogeveen et al., 2001). To the best of our knowledge, there are no data available on AMS milking routines applying different water temperatures for teat cleaning.

This study was designed to test the hypothesis that the routine of premilking teat cleaning influences OT release and milk removal during milking in AMS. Premilking routines included different duration of cleaning and different temperatures of the water used for cleaning.

MATERIALS AND METHODS

Animals

Red Holstein/German Fleckvieh crossbreed cows (average yield: 7800 kg/yr) were investigated during their

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Table 1. Descriptive statistics of premilking teat preparation phases in automatic milking systems.

Trait	Treatment							
	T0 ¹		T1 ²		T1C ³		T2 ⁴	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Time to attach first teat cup (s)	13.1	0.4	13.9	0.4	14.5	0.51	3.3	0.4
Time to attach all 4 teat cups (s)	55.7	1.0	52.5	0.6	53.4	0.8	54.2	0.9
Preparation lag time (s)	...		81.5	0.6	82.0	0.6	145.2	1.0
Cleaning time	...		57.9	0.4	58.9	1.0	122.3	0.9

¹T0 = Treatment without premilking teat preparation.

²T1 = Cycle of teat cleaning with warm water (30 to 32°C), standard routine.

³T1C = Cycle of teat cleaning with cold water (13 to 15°C).

⁴T2 = Cleaning cycles with warm water (30 to 32°C).

voluntary milkings in experiments 1 and 2. Cows were designated to 3 lactational stages: early (<100 d), mid (100 to 200 d), and late (>200 d) lactation. All cows were routinely milked in AMS (VMS, DeLaval, Tumba, Sweden) with guided cow traffic, where cows could only reach the feeding area by passing the AMS (Harms et al., 2002). The minimum milking interval for all cows was 5 h. If cows did not visit the AMS voluntarily, they were manually driven to the AMS based on their production levels (cows with milk yield less than 20 kg/d after an interval of 12 h, cows with milk yield between 20 and 30 kg/d after an interval of 11 h, and cows producing more than 30 kg/d after an interval of 10 h from previous milking). Teat preparation, with a special teat cup just for teat cleaning, consisted of teat cleaning, removal of foremilk, and teat drying for 9 s for each teat. For teat cleaning, a mixture of compressed air and water was applied for 5 s. Foremilk was collected by application of a vacuum in the teat cup at the end of teat cleaning. Drying off the teat was accomplished by blowing air between the liner and the teat during the removal of the teat cup. One cleaning cycle consisted of cleaning all 4 teats at once. Teat cups of each quarter were removed individually when milk flow dropped below 100 g/min for 5 s. Concentrate was offered to all cows during milking according to their individual milk production. Cows with a milk production of less than 22 kg/d received a maximum of 1 kg/d of concentrate, whereas cows producing more than 22 kg/d received 1.5 kg/d of concentrate. All animals were kept indoors, and were fed a mixed roughage ration (65% maize silage and 35% grass silage) ad libitum at the forage lane. In the stable, cows were offered concentrate from 2 automatic feeders depending on their production levels (at least 0.5 kg/cow per day and up to 10.5 kg/cow per day).

Experiment 1

Sixty-two cows in their first to fifth lactations were randomly assigned to 4 treatments in crossover design

during 3 recording periods over 8 mo. Each recording period lasted for 4 d. Treatments were randomly changed every 24 h. Treatments of premilking teat preparation were no premilking teat preparation (T0); 1 cleaning cycle with warm water (30 to 32°C; standard routine; T1), one cleaning cycle with cold water (13 to 15°C; T1C); or 2 cleaning cycles with warm water (30 to 32°C; T2). Quarter milk flow was recorded during all experimental milkings with a specially rebuilt set of 4 Lactocorders (WMB AG, Balgach, Switzerland).

Experiment 2

Ten cows with no clinical signs of disease were used for blood sampling during one experimental period. Five cows were primiparous, 3 cows were in their second, 1 in its third, and 1 in its fourth lactation. Two cows were in early stage of lactation, 76 ± 4 DIM, with an average milk production of 2.53 ± 0.1 kg/h; 4 in mid lactation, 161 ± 5 DIM, with an average milk production of 1.91 ± 0.1 kg/h; and 4 in late lactation, 255 ± 7 DIM, with an average milk production of 1.19 ± 0.1 kg/h. Treatments T0, T1, T1C, and T2 were randomly assigned to 10 cows during the experimental period in a crossover design. The experimental period consisted of a catheterization day and 4 additional experimental days. One week prior to the start of experiment, cows were habituated to the experimental settings by getting them adapted to the presence of people and technical equipment and by touching their neck during milking to mimic handling of a permanent catheter. Blood samples were obtained through a permanent catheter that was inserted in a jugular vein the day before the start of experiment. Blood samples were taken every 30 s during premilking teat preparation and the first 2 min of milking. Thereafter, blood samples were taken every minute until the end of milking. Blood samples were treated with EDTA to prevent coagulation, cooled on ice, and centrifuged at $1500 \times g$ for 15 min. Blood plasma was stored at -20°C until OT concentrations were determined by radioimmu-

noassay (Schams, 1983). Quarter milk flow was recorded as in experiment 1.

Data Analyses

Data of milkings with unsuccessful attachment within 1 min from the start of teat cup attachment, reattachment of the teat cups during milking, incomplete milking (quarter milk yield was less than 50% of expected quarter milk yield at a particular milking), and technical failure caused by the AMS (in total 8% of the milkings) were excluded from the analyses. Preparation lag time was the time from the start of teat cleaning until the first teat cup was attached. Bimodality of milk flow was detected if any of the quarter milk flow curves had a flow pattern with 2 increments separated by a clear drop in milk flow for more than 200 g/min within 1 min after the start of milking. Start of alveolar milk ejection was defined as the start of the second increment in bimodal milk flow curves from the time when the first teat cup was attached. Main milking time was defined as the time between the attachment of the first teat cup and the removal of the last teat cup. Total milking time was defined as main milking time plus premilking teat preparation. In experiment 1, the following model was used:

$$Y_{ijklm} = \mu + T_i + L_j + D_k + I_l + \text{Cow}_m \cdot T_i(L_j) + I_l^2 + T_i \cdot D_k + T_i \cdot I_l + T_i \cdot I_l^2 + T_i \cdot D_k \cdot I_l + e_{ijklm} \quad [1]$$

where

Y_{ijklm} = milk production (kg/h), or average flow rate (kg/min), or peak flow rate (kg/min), or main milking time (min), or milk yield (kg), or start of alveolar milk ejection (s),

μ = overall mean,

T_i = effect of treatment ($i = 1$ to 4),

L_j = effect of lactation class ($j = 1$ to 5),

D_k = effect of lactation stage ($k = 1$ to 3),

I_l = effect of milking interval ($l = 1$ to 20 h),

$\text{Cow}_m \cdot T_i(L_j)$ = random effect of cow ($m = 1$ to 62) and treatment interaction within lactation class (error term for testing treatment effects), and

e_{ijklm} = residual error.

In experiment 2, the following model was used:

$$Y_{ij} = \mu + T_i + \text{Cow}_j(T_i) + e_{ij} \quad [2]$$

where

Y_{ij} = OT values,

μ = overall mean,

T_i = fixed effect of treatment ($i = 1$ to 4),

$\text{Cow}_j(T_i)$ = random effect of cow ($j = 1$ to 10) within treatment (error term for testing treatment effects), and

e_{ij} = residual error.

Pairwise differences between treatment means were tested by using the Tukey-Kramer test with multiple comparison adjustment. The analyses were performed by using the MIXED procedure of SAS (SAS Institute, 1999).

RESULTS

Experiment 1

Milking characteristics were evaluated from the data set of 829 milkings. The interval between milkings was 9.6 ± 0.2 h in early, 10.0 ± 0.2 h in mid, and 10.5 ± 0.2 h in late lactation. Bimodal curves ($n = 151$) demonstrating alveolar milk ejection after removal of the cisternal milk were detected only in treatment T0. Occurrence of the main milk flow was observed in all treatments with premilking teat preparation after 22 ± 1 s. Time needed to attach the first teat cup, with or without premilking teat preparation, ranged from 13 to 15 s (Table 1). Time to attach all 4 teat cups ranged from 53 to 56 s. Preparation lag time in treatments T1, T1C, and T2 was 82, 82, and

Figure 1. Mean average flow rate in different milking intervals for treatment without premilking teat preparation (T0, ●); one cycle of teat cleaning with warm water (30 to 32°C), normal routine (T1, ◆), 1 cycle of teat cleaning with cold water (13 to 15°C) (T1C, ▲); or 2 cleaning cycles with warm water (30 to 32°C) (T2, ■). Differences between the treatments are indicated by 2 asterisks ($P < 0.01$) and one asterisk ($P < 0.05$).

Table 2. Milking characteristics in different premilking teat preparation in experiment 1. Least square means $\pm$ SEM.

Milking characteristic	Treatment				SEM	I ⁵	T $\times$ I
	T0 ¹	T1 ²	T1C ³	T2 ⁴			
Milking time							
Main (min)	5.59 ^a	5.10 ^b	5.00 ^b	4.92 ^b	0.2	***	
Rear left (min)	4.90 ^a	4.39 ^b	4.24 ^b	4.19 ^b	0.2	***	
Rear right (min)	5.10 ^a	4.35 ^b	4.23 ^b	4.19 ^b	0.2	***	
Front left (min)	3.36	3.23	3.21	3.18	0.2	***	
Front right (min)	3.65	3.36	3.34	3.29	0.2	***	
Milk yield							
Total (kg)	10.68	10.61	10.52	10.37	0.3	***	
Rear left (kg)	3.36	3.34	3.29	3.26	0.1	***	
Rear right (kg)	3.08	3.14	3.11	3.05	0.1	***	
Front left (kg)	2.02	1.99	2.00	1.93	0.1	***	
Front right (kg)	2.22	2.14	2.12	2.13	0.1	***	
Milk production (kg/h)	1.19	1.19	1.18	1.16	0.04		
Peak flow rate (kg/min)	4.13	4.11	4.19	4.15	0.2	***	
Average flow rate (kg/min)	1.96 ^a	2.15 ^b	2.18 ^b	2.16 ^b	0.08	***	*

^{a,b}Least square means with different superscripts differ ($P < 0.05$).

¹T0 = Treatment without premilking teat preparation.

²T1 = 1 cycle of teat cleaning with warm water (30 to 32°C), standard routine.

³T1C = 1 cycle of teat cleaning with cold water (13 to 15°C).

⁴T2 = 2 cleaning cycles with warm water (30 to 32°C).

⁵I = Milking interval.

* $P < 0.05$.

*** $P < 0.001$.

145 s, respectively. Main milking time was significantly longer ($P < 0.05$) without premilking teat preparation as compared to all other treatments (Table 2). Quarter milking time without premilking teat preparation was numerically longer in front quarters and significantly longer ($P < 0.05$) in rear quarters than in all other treatments. Milk production, peak flow rate, and total and quarter milk yields did not differ between the treatments. Average flow rate was significantly lower ($P < 0.05$) without premilking teat preparation than in all other treatments (Table 2). Milking interval explained most of the variation for all measured milking characteristics in the model [1] ($P < 0.001$). Average milk flow was significantly lower up to 8 h from the previous milking in treatment without premilking teat preparation compared with all other treatments (Figure 1). Time until start of alveolar milk ejection without premilking teat preparation was decreasing up to 11 h from the previous milking ($P < 0.05$) (Figure 2). Numerically the shortest time until occurrence of alveolar milk ejection without premilking teat preparation occurred in early lactation up to milking intervals of 10 h. Thereafter, no significant differences of occurrence of the alveolar milk ejection were observed between lactational stages without premilking teat preparation. Time until alveolar milk ejection without premilking teat preparation was shorter after a long milking interval than after a short milking interval (68 vs. 102 s, respectively).

Experiment 2

Oxytocin concentrations were measured during 69 milkings in 10 cows. Oxytocin baseline level was between 4.7 and 5.6 ng/L (Table 3). Before the start of teat cup attachment, the OT concentration was lower ($P < 0.05$) in T0 than in the other treatments. Oxytocin concentrations

Figure 2. Time until alveolar milk ejection in bimodal curves without premilking teat preparation. Means $\pm$ SEM. ^{abcd}Means without common superscript are significantly different ($P < 0.05$).

Table 3. Least square means and SEM of effects of treatments on oxytocin release.

Oxytocin, ng/L	Treatment							
	T0 ²		T1 ³		T1C ⁴		T2 ⁵	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Start of cleaning	5.1	0.4	5.2	0.4	4.7	0.4	5.6	0.4
Start of teat cup attachment	6.2 ^a	0.8	103.8 ^b	16.1	76.7 ^b	15.5	100.0 ^b	17.4
30 s after first teat cup attached	94.9	18.2	101.8	16.9	76.7	16.3	80.4	18.3
1 min of milking (AUC ¹ /min)	76.7	14.6	96.8	13.4	83.4	12.7	92.6	14.2
2 min of milking (AUC ¹ /min)	82.6	13.6	91.3	12.4	81.8	11.7	85.8	13.1
Entire milking (AUC ¹ /min)	67.3	9.8	67.8	9.0	58.7	8.6	62.6	9.7

^{a,b}Least square means with different superscripts differ ($P < 0.05$).

¹AUC = Area under the curve.

²T0 = Treatment without premilking teat preparation ($n = 15$).

³T1 = 2 cycles of teat cleaning with warm water (30 to 32°C), standard routine ($n = 18$).

⁴T1C = 1 cycle of teat cleaning with cold water (13 to 15°C) ($n = 20$).

⁵T2 = 2 cleaning cycles with warm water (30 to 32°C) ($n = 16$).

rose markedly within only 30 s after teat cup attachment started without premilking teat preparation. Peak OT concentrations were observed during premilking teat preparation in treatments T1, T1C, and T2, and at 30 s of milking in treatment T0. Oxytocin release remained elevated above baseline concentrations throughout milking and did not differ between any of the treatments (Figure 3).

DISCUSSION

Fast and complete milk removal requires milk ejection in response to teat stimulation. Premilking teat cleaning is supposed to induce milk ejection in AMS milking during the teat cleaning process. Moreover, the lag time to attachment of the first teat cup with premilking teat cleaning is usually longer in AMS than in conventional milking. Rasmussen et al. (1992) reported optimal preparation lag time in conventional milking to be 60 to 90 s; when it is longer than 3 min more residual milk and lower overall milk yields occur. A delay of 2 min after the end of a 1-min premilking teat preparation (i.e., preparation lag time of 3 min) transiently reduced OT concentrations, increased residual milk fraction, and reduced main milk yield (Bruckmaier et al., 2001). Preparation lag times in our study were not longer than 2.5 min and no negative effect on milk yield was observed. Obviously, a delay between premilking teat preparation and attachment of the first teat cup (i.e., the start of milking) is more crucial for milking performance than the duration of premilking teat preparation. If premilking teat preparation is longer than 20 s, addition of fore stripping to the milking routine did not influence time to milk flow, machine on time, average milk flow, or milk yield (Rasmussen et al., 1992). Although main milking time is shortened when prestimulation is applied, total milking

time (main milking time plus premilking teat preparation time) is usually not reduced (Rothenanger et al., 1995). In this study, main milking time was longer in treatment without premilking teat preparation than in other treatments, most evident in the rear quarters. One or 2 cleaning cycles of premilking teat preparation compared with no premilking preparation did not reduce total milking time and showed no differences in milking characteristics. This finding suggests that the use of two instead of one cleaning cycle would only decrease the capacity of the AMS, without beneficial effect on milk ejection. Little or no cisternal milk is present in the udder until 4 h after the previous milking (Knight et al., 1994). The alveolar milk fraction is shifted into the cisternal cavities during milk ejection. The alveolar milk ejection is delayed after short milking intervals and in late lactation because more time is needed for milk to be ejected into the milk ducts and cisternae from the partially filled alveoli (Bruckmaier et al., 2001). In the present study, short milking intervals (5 to 7 h) prolonged the time to alveolar milk ejection without premilking teat preparation. It seems likely that after 8 h from the previous milking, there is sufficient cisternal milk to prevent a transient drop in the milk flow while alveolar milk ejection occurs, independent of lactational stage. Prolonged main milking time, caused by the delayed occurrence of alveolar milk ejection at milking without premilking teat preparation, diminished the average flow rate up to a milking interval of 8 h.

Basal OT concentrations were low in all treatments. In the present study, OT concentrations reached their peak within 30 s from the start of stimulation in all treatments (in T0 from the start of teat cup attachment). Peak concentration lasted for 1 to 2 min and thereafter decreased gradually, while remaining above baseline until the end of milking. Oxytocin release during milking

Figure 3. Oxytocin profiles, release during milking, total and quarter milk flow in one cow. T0 = milking routine without premilking teat preparation. T1 = milking routine with one cycle of teat cleaning with warm water ($>30^{\circ}\text{C}$). T1C = milking routine with one cycle of teat cleaning with cold water ($<15^{\circ}\text{C}$). T2 = Milking routine with 2 cleaning cycles with warm water ($>30^{\circ}\text{C}$). Teat cup or first teat cup ($\uparrow$) attachment and teat cup or last teat cup ($\downarrow$) removal in quarter and total milk flow curve. Duration (■) of premilking teat preparation.

(area under the curve/min) was similar with those measured after a milking interval of 12 h in conventional milking (Bruckmaier and Hilger, 2001) and in AMS milking (Hopster et al., 2002; Macuhova et al., 2003). Premilking teat preparation of 1 or 2 cleaning cycles prevented delayed milk ejection and no bimodal milk flow curves, indicating delayed milk ejection occurred. Milking without premilking teat preparation induce OT release within the first minute of milking, causing delayed milk ejection, prolonged main milking time, and reduced average flow rate (Zinn et al., 1982; Mayer et al., 1984).

Cleaning water temperature close to body temperature (around 40°C) compared to cold water (10°C) did not influence milking characteristics during premilking teat preparation (Dodd and Foot, 1947; Frommholdt, 1973). On the contrary, Philips (1984) found a significantly longer premilking teat stimulus requirement with cold (15°C) rather than with hot water (54°C). A part of this difference could be assigned to the postulated longer premilking stimulus required by Jersey compared with the Holstein breed (Rasmussen et al., 1992). Hogewerf et al. (1998) showed that the intensity of the pulsating water stream in the teat cup had crucial influence on the milk flow rates, whereas milk yield remained unchanged. In this study no differences of OT release, milk ejection, and milk removal were observed between milking routines with cold and warm water. Moreover, no kicking of the teat cup or discomfort for the cow was observed during milking with cold or warm water. It seems likely that the intensity and duration of the stimulus during premilking teat preparation are more important than the temperature of the water applied. Therefore, a reduction of water temperature could reduce energy costs for water heating in AMS dairy herds.

CONCLUSIONS

The teat cleaning device used in the AMS is suitable to induce OT release and milk ejection before the start of milking. Similar effects have been demonstrated with warm and cold water. A milking routine with 1 or 2 cleaning cycles (i.e., premilking teat preparation of 58 to 60 and 122 s) induced OT release and milk ejection, independent from the milking interval or stage of lactation. Alveolar milk ejection occurs about 1 min from the start of teat stimulation by liner after longer milking intervals or up to 2 min after short milking intervals. Adequate premilking teat preparation time should be applied to the cows at milking intervals shorter than 8 h.

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