J. Dairy Sci. TBC
https://doi.org/10.3168/jds.2024-24692

Estimation of udder emptying based on milk constituents of strip samples after milking

Benjamin Jenni, Olga Wellnitz, and Rupert M. Bruckmaier*
Veterinary Physiology, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland

ABSTRACT

Milk ejection disorders were induced by oxytocin receptor blockade. We tested the hypothesis that the degree of udder emptying at incomplete milk ejection can be estimated based on the concentration of various milk constituents in different milk fraction samples. To induce different levels of spontaneous udder emptying (SUE) 10 Holstein dairy cows were milked either with or without i.v. injection of the oxytocin receptor blocking agent atosiban (ATO). In ATOearly, 12 µg/kg BW ATO was injected immediately before and in ATOlate directly after a 1 min manual udder preparation. The normal milking routine served as Control treatment. In all 3 treatments the udder was completely emptied by the i.v. injection of 10 IU oxytocin (OT) at the end of spontaneous milk flow. During all experimental milkings 4 milk samples were taken in all treatments: at the start of udder preparation (foremilk; FM), immediately after cessation of spontaneous milk flow and cluster detachment by hand stripping (strip milk; SM), from spontaneous removed milk in the bucket 1 (milk before OT; MBOT) and from the milk obtained after OT injection in the bucket 2 (milk after OT; MAOT). Fat, protein, lactose and electrolytes (Na, Cl and K) were measured in each milk sample. In addition, electrical conductivity (EC) was determined in parallel to continuous milk flow recording. The treatments induced individual degrees of SUE; therefore, the final evaluations of data were based on SUE classes instead of treatments. The most pronounced differences of milk constituents at different degrees of SUE were found for the milk fat content. The fat content of SM and MBOT remained almost unchanged up to 60% SUE, but was considerably higher if >80% of the milk was spontaneously removed. The concentrations of Na and Cl were highest and of K lowest if less than 20% on milk was received in the different samples. The EC was higher in SM and MBOT if <20% of milk was received. In conclusion, the blockade of the OT effect influences primarily the fat content, which confirmed an OT-induced fat secretion during milking. Similar effects are likely found in situations of disturbed milk ejections, caused by a lacking or reduced release of OT in response to different degrees of tactile udder stimulation. Our results show that the measurement of fat content and the EC in strip milk samples collected after cluster detachment can be used to estimate the completeness of udder emptying.
Keywords: atosiban, oxytocin, fat content, strip milk samples, udder emptying

INTRODUCTION

Complete milk removal during machine milking is essential for maintaining high milk production and udder health in dairy cows. Only the cisternal fraction of the stored milk in the udder is immediately available for milking without milk ejection. The main portion of milk, the alveolar fraction accounting to at least 80%, is fixed by capillary forces in small ducts and alveoli, and can only be removed after milk ejection. This active transfer of alveolar milk into the cisternal cavities requires the release of oxytocin (**OT**) from the posterior pituitary through tactile teat stimulation (Bruckmaier and Blum, 1998). Disturbance of the milk ejection reflex is caused by lacking or reduced release of OT in response to tactile udder stimulation and leads to incomplete udder emptying (Bruckmaier and Blum, 1998). Disturbance of milk ejection has been reported under various conditions such as unfamiliar surroundings (Bruckmaier et al., 1997), primiparous cows immediately after calving, multiparous cows in different stage of lactation (Belo et al., 2009), during peak estrus and after changing from calf suckling to machine milking (Tančin et al., 1995). Also, a management of both suckling and machine milking such as in cow-calf contact rearing systems has been reported to cause inhibition of oxytocin release and milk ejection at machine milking (Kaskous et al., 2006). However, precise studies includ-

Received January 19, 2024.
Accepted February 28, 2024.
*Correspondence: Prof. Rupert M. Bruckmaier, Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3012 Bern, Switzerland, *Phone:* +41-31-684 23 24, *E-mail*: rupert.bruckmaier@unibe.ch

ing measurements of harvested vs. residual milk or circulating OT concentrations in cow-calf contact rearing systems are scarce. Mostly observational results were based on the bulk milk fat content which appears to be reduced if milk ejection is incomplete (Zipp et al., 2018; Barth, 2020). But the quantitative relation of reduced fat content and reduced spontaneously removed milk (the amount of milk that could be removed by the milking machine without oxytocin injection) is not known. Previous studies have shown that there are changes of the contents of milk constituents in different milk fractions, especially the fat content increases steadily during the course of milking (Ontsouka et al., 2003; Sarikaya et al., 2005). More recently it was detected in mice that lipid droplets were released after the OT-induced contraction of myoepithelium (Masedunskas et al., 2017). A similar effect is very likely also the reason for compositional changes in the milk of dairy cows.

Therefore, we tested the hypothesis that changes in milk composition, mainly the fat content, occur during inhibited milk ejection through oxytocin receptor blockade. Milk composition may help to detect and to estimate the level of incomplete udder emptying at machine milking caused by lacking or reduced oxytocin release in practical farming systems such as cow-calf-contact rearing systems.

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 2022–02-FR).

Animals and Housing

Ten Holstein dairy cows from the Swiss Federal Research Station Agroscope Posieux were enrolled in this study. As only the 10 cows were available and results were not predictable, no sample size calculation was performed. At the start of experimental milkings, cows were between 211 and 346 DIM of their second (n = 5), third (n = 2), fourth (n = 1), and sixth (n = 2) lactation. The 305-d milk production of the experimental cows in their previous lactation ranged from 7,922 to 9,987 kg (8,850 ± 350 kg). All cows passed a general health check (rectal temperature, breathing rate, free of lameness and injuries) before the experiments and were free of signs of clinical mastitis or other diseases. The cows were kept in loose housing between milkings. Experimental milkings were performed in a familiar tie-stall barn. Cows were fed a mixed ration consisting of corn silage, grass silage, and aftermath, which was supplemented by minerals. Additional concentrate was fed according to their individual production levels.

Experimental Protocol

Experimental milkings were performed with a bucket milker during afternoon milkings (11 h interval from previous morning milking). The system vacuum was set at 41 kPa, the pulsation rate was 60 cycles per minute at a pulsation ratio of 65/35. The milking cluster consisted of a Uniflow claw (SACMilking, Kolding, Denmark), Uniflow teat cups (product number 21214.817, SACMilking) with square Uniflex liners (product number 13522, SACMilking).

The milking routine consisted of a 60 s udder preparation, which included foremilk sampling within 20 s, teat cleaning with disposable disinfectant cloths, and teat stimulation for the remaining time. Milk flow was recorded during all experimental milkings using a portable electronic recording unit (LactoCorder, WMB AG, Balgach, Switzerland), and the exact amount of milk for each milk fraction in the bucket was additionally determined with weighing scales. After each experimental milking, teat dipping was performed with an iodine-based agent (VelouCid D, Ecolab, St. Paul, USA).

Milkings were performed either without or with i.v. administration of the oxytocin receptor blocking agent ATO (Ferring Research Institute AB, Malmö, Sweden) in a randomized order. It was shown that ATO has a half-life of 18 min in cows (Wellnitz et al., 1999). For i.v. injections, an indwelling catheter (Intranule, 105 mm and 1.9 × 2.4 mm, 13 G, VYGON, Ecouen, France) was inserted in one jugular vein approximately 1 h before milking. Three different treatments were conducted to induce different levels of udder emptying (**UE**). In **ATOearly**, 12 μg/kg BW ATO in 10 mL sterile saline solution was injected immediately before and in **ATOlate** directly after the 1-min manual udder preparation. The **Control** treatment consisted of the normal milking routine without administration of ATO. In all 3 treatments, the cluster was detached after cessation of spontaneous milk flow, followed by the i.v. injection of 10 IU OT (Oxytocin Stricker, Werner Stricker AG, Zollikofen, Switzerland), and re-attachment of the cluster for complete udder emptying. For separate recording and sampling of spontaneously removed milk and milk harvested in response to OT, the respective milk fractions were collected in different buckets (bucket 1 and bucket 2, respectively). All cows (n = 10) received all 3 treatments. Each treatment was performed once at a cow-individual randomized sequence of treatments to prevent treatment x test day confounding. Between the experimental milkings the cows were milked 3 times in the milking parlor within the normal milking routine of the farm. These milkings served as "washout" from the previous experimental milking and to prevent any potential desensitization to OT (Belo and Bruckmaier, 2010).

Milk Sampling and Analyses

During each experimental milking 4 milk samples were taken in all treatments. The first sample was taken at the start of udder preparation merged from ~15 mL samples per quarter (foremilk; **FM**). Immediately after cluster detachment milk (~15 mL of each quarter merged) was collected by hand stripping (strip milk; **SM**) and a proportional milk sample was taken from spontaneously removed milk after careful stirring the milk in bucket 1 (milk before OT; **MBOT**). Another proportional milk sample was taken from the milk obtained after OT injection after careful stirring the milk in bucket 2 (milk after OT; **MAOT**). The milk samples for gross composition analysis were stabilized with bronopol, stored in a refrigerator (5°C) and sent to a commercial laboratory (Suisselab AG, Zollikofen, Switzerland) for analysis of fat, protein and lactose using a MilkoScan RM 7 / FT 6000 (Foss electric, Hilleroed, Denmark). Milk for electrolyte (Na, Cl and K) measurements was frozen at −20°C until analysis. After thawing milk serum was obtained by 2-step centrifugation at 1,500 x g for 15 min at 4°C and then at 20,000 x g for 45 min at 4°C. Electrolytes were measured with the autoanalyzer (Cobas Pure c303, Roche, Basel, Switzerland) using ion-selective electrodes.

The electrical conductivity (**EC**) of the milk samples was determined by means of the EC recording of the LactoCorder. We defined the EC of FM as the EC of the first milk which reached the LactoCorder at the start of milking, EC of SM as the last value before abrupt EC decrease due to empty LactoCorder at the end of milking, EC of MBOT as the arithmetic mean of the values between EC of FM and EC of SM, and the EC of MAOT as the arithmetic mean of the values of the milking after cluster reattachment (between steep increase and steep decrease of EC, same as for MBOT, respectively).

Statistical Analysis

The SAS software was used (version 9.4, SAS Institute Inc., North Carolina, USA) for statistical analysis. Data are presented as arithmetic means and standard errors of the respective means. For the determination of degree of UE, the sum of removed milk before OT and removed milk after OT was calculated (total milk yield; **TMY**), which was assumed to be 100% UE. The percentage of removed milk before OT to TMY (100% UE) resulted in UE of spontaneously removed milk for each milking (spontaneous udder emptying; **SUE**). The milkings were classified treatment independently in SUE-Groups: < 20% SUE, 20 – 40% SUE, 40 – 60% SUE and >80% SUE. No milking at SUE of 60 – 80% occurred. Therefore, this class could not be included. Changes in milk constituents during the course of milking were tested based on the SUE-Groups. Statistical testing was performed based on LSMEANS using the mixed procedure (PROC MIXED) with the Tukey-Kramer adjustment for multiple comparisons. The model included SUE-Group as fixed effect and cow as random subject. Effects were considered to be significant when $P < 0.05$. For milk constituents with the most pronounced differences between SUE-Groups we have used a quadratic regression analysis (SigmaPlot 12.5) to estimate the degree of SUE.

RESULTS

Milk removal and degree of udder emptying

The spontaneously removed milk before OT was 2.26 ± 0.65 kg, 5.81 ± 0.79 kg and 10.96 ± 0.66 kg. This corresponds to 18.42 ± 4.89%, 46.02 ± 5.17% and 91.38 ± 1.91% of udder emptying in ATOearly, ATOlate and Control, respectively. After OT injection, 9.64 ± 0.63 kg milk was removed in ATOearly, 6.75 ± 0.77 kg in ATOlate and 1.08 ± 0.27 kg in Control. The TMY did not differ among treatments and was 11.90 ± 0.41 kg in ATOearly, 12.56 ± 0.56 kg in ATOlate and 12.03 ± 0.78 kg in Control. The degree of SUE differed considerably among cows, both within and between ATO treatments, ranging from 2.87 to 59.08%, 13.23 to 87.83%, and 83.16 to 97.53% in ATOearly, ATOlate and Control, respectively (Figure 1). We classified the milkings based on SUE and independent of the individual reaction to one of the treatments in 4 SUE-Groups.

Milk constituents

Means of milk constituents in different milk fraction samples at different degrees of SUE are presented on Table 1. In FM, milk gross composition (fat, protein and lactose), milk electrolytes (Na, Cl, K) and EC did not differ among SUE-Groups. Therefore, we added FM means ± SEM of all SUE-Groups up.

Concentration of fat in MBOT, SM and MAOT was higher in >80% SUE than in all other SUE-Groups ($P < 0.05$). In MAOT the concentration of fat in 20 – 40% and 40 – 60% SUE was higher than in <20% SUE ($P < 0.05$). Figure 2 shows the milk fat content of MBOT

Table 1. Milk constituents (means ± SEM) in different samples at different degrees of spontaneous udder emptying

			Spontaneous udder emptying[1]				
Parameter	Unit	Sample[2]	<20%	20 – 40%	40 – 60%	>80%	FM[3]
Observations	n		9	7	7	11	34
Fat	g/100g	MBOT	2.89 ± 0.29^{b}	2.05 ± 0.31^{b}	2.23 ± 0.18^{b}	4.21 ± 0.16^{a}	2.34 ± 0.16
		SM	2.96 ± 0.28^{b}	2.83 ± 0.28^{b}	2.82 ± 0.10^{b}	6.46 ± 0.33^{a}	
		MAOT	5.17 ± 0.22^{c}	6.35 ± 0.30^{b}	7.64 ± 0.68^{b}	9.70 ± 0.69^{a}	
Protein	g/100g	MBOT	3.76 ± 0.11^{b}	4.04 ± 0.14^{a}	3.78 ± 0.14^{ab}	3.82 ± 0.11^{b}	3.78 ± 0.06
		SM	3.83 ± 0.09^{ab}	3.92 ± 0.13^{a}	3.70 ± 0.14^{ab}	3.64 ± 0.11^{b}	
		MAOT	3.89 ± 0.10^{a}	3.87 ± 0.15^{ab}	3.54 ± 0.13^{b}	3.43 ± 0.11^{c}	
Lactose	g/100g	MBOT	4.22 ± 0.12^{b}	4.74 ± 0.07^{a}	4.71 ± 0.08^{a}	4.71 ± 0.04^{a}	4.17 ± 0.08
		SM	4.10 ± 0.21^{b}	4.51 ± 0.07^{a}	4.45 ± 0.16^{ab}	4.29 ± 0.10^{ab}	
		MAOT	4.69 ± 0.06^{a}	4.68 ± 0.07^{a}	4.58 ± 0.08^{a}	4.34 ± 0.04^{b}	
Na	mmol/L	MBOT	37.81 ± 3.37^{a}	25.34 ± 1.97^{b}	24.31 ± 1.44^{b}	21.09 ± 0.78^{b}	40.68 ± 2.54
		SM	41.72 ± 7.00^{a}	32.23 ± 2.85^{ab}	31.57 ± 4.21^{ab}	30.76 ± 3.69^{b}	
		MAOT	20.68 ± 0.63^{b}	20.04 ± 0.99^{b}	20.51 ± 0.66^{b}	26.04 ± 1.87^{a}	
Cl	mmol/L	MBOT	39.78 ± 1.85^{a}	31.73 ± 1.65^{b}	34.10 ± 1.20^{b}	29.34 ± 0.66^{b}	42.43 ± 1.42
		SM	42.26 ± 3.74^{a}	35.97 ± 1.51^{ab}	39.23 ± 3.18^{ab}	35.71 ± 2.01^{b}	
		MAOT	29.33 ± 1.05^{ab}	28.43 ± 1.58^{b}	31.67 ± 1.11^{ab}	33.36 ± 1.13^{a}	
K	mmol/L	MBOT	31.40 ± 2.35^{b}	37.08 ± 1.66^{a}	39.12 ± 1.22^{a}	38.52 ± 1.30^{a}	31.25 ± 1.10
		SM	32.02 ± 2.92	34.37 ± 1.98	36.07 ± 1.61	34.96 ± 1.81	
		MAOT	41.55 ± 1.60^{a}	38.30 ± 1.84^{ab}	40.37 ± 1.32^{ab}	37.75 ± 1.56^{b}	
EC[4]	mS/cm	MBOT	6.76 ± 0.18^{a}	6.03 ± 0.19^{b}	6.15 ± 0.19^{b}	5.66 ± 0.13^{b}	6.28 ± 0.10
		SM	6.90 ± 0.21^{a}	6.20 ± 0.18^{b}	6.33 ± 0.30^{b}	5.78 ± 0.16^{c}	
		MAOT	5.51 ± 0.13	5.34 ± 0.15	5.35 ± 0.18	5.58 ± 0.14	

[a, b, c] No common letter indicates a significant difference ($P < 0.05$) between SUE-Groups.

[1]Spontaneous udder emptying: The milkings were classified treatment independently in SUE-Groups.

[2]Milk samples: MBOT = milk before OT; SM = strip milk; MAOT = milk after OT.

[3]Foremilk: added Means ± SEM of the SUE-Groups.

[4]EC = electrical conductivity.

(A), SM (B) and MAOT (C) relative to SUE. Protein concentration in MBOT was higher in 20 – 40% SUE than in 0 – 20% and >80% SUE ($P < 0.05$). In SM the concentration of protein was higher in 20 – 40% SUE than in >80% SUE ($P < 0.05$). The concentration of protein in MAOT was higher in <20% SUE than in 40 – 60% SUE and >80% SUE ($P < 0.05$), and reached the lowest values in >80% SUE ($P < 0.05$). Lactose in MBOT and SM was lowest in <20% SUE, reached higher values with further SUE in MBOT ($P < 0.05$) and was highest at 20 – 40% SUE in SM ($P < 0.05$). In MAOT lactose reached the lowest concentration in >80% SUE ($P < 0.05$). Table 2 shows the differences in the concentration of the milk constituents between SUE-Groups in the respective sample. These changes are indicated by arrows for better understanding of directions of changes based on our results but indicate that absolute values may vary between individuals, breeds, or milking procedures.

Na and Cl in MBOT was highest in <20% SUE and reached lower values with further SUE ($P < 0.05$). The K concentration in MBOT was lower ($P < 0.05$) in <20% SUE than in all other SUE-Groups. The concentration of Na and Cl in <20% SUE of the SM sample was higher than in >80% SUE ($P < 0.05$). In SM there were no changes in K concentration between SUE-Groups. Na und Cl concentration in MAOT reached the highest values in >80% SUE. This Na concentration was higher than in all other SUE-Groups ($P < 0.05$). The Cl concentration in >80% SUE was higher than

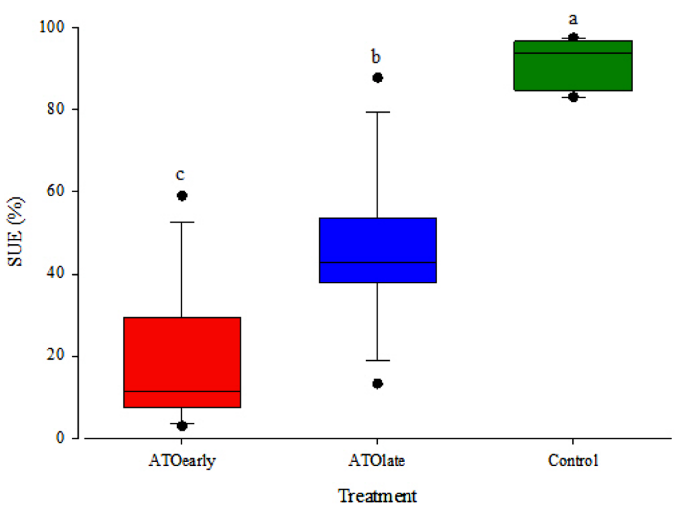

Figure 1. The degree and the variation of spontaneous udder emptying (SUE) of the atosiban injection immediately before (ATOearly) and directly after (ATOlate) the 1 min udder preparation compared with control milkings (Control). [a, b, c] No common letter indicates a significant difference ($P < 0.05$) between treatments.

in 20 – 40% SUE ($P < 0.05$). In MAOT K reached the lowest concentration in >80% SUE, this concentration was lower than in <20% SUE ($P < 0.05$). EC in MBOT was higher in <20% SUE than in all other SUE-Groups ($P < 0.05$). EC in SM was highest in <20% SUE ($P < 0.05$) and was lowest in >80% SUE ($P < 0.05$). In MAOT EC did not differ between SUE-Groups.

Quadratic regression analysis

The highest coefficient of determination was found for fat in SM ($R^2 = 0.82$). The resulting equation was fat % = 3.33 - 0.054 * SUE + 0.001 * SUE^2.

DISCUSSION

In the present study, we aimed to induce different levels of incomplete milk ejection by the i.v. injection of the oxytocin receptor blocking agent ATO immediately before or directly after a 1-min manual udder stimulation, followed by a complete emptying in response to OT injection. We investigated the changes in milk composition and the suitability of the concentration of various milk constituents in different milk fractions to estimate the degree of udder emptying caused by incomplete milk ejection during milking as compared with control milkings.

Effects of ATO and OT injections

The injection of ATO lead to a preterm cessation of milk flow. If ATO was injected before manual udder preparation (ATOearly) only small amounts <20% of the total milk were spontaneously available. In agreement with our earlier studies the obtained milk represented in most cases the cisternal milk fraction likely because OT released in response to teat stimulation could not bind to OT receptors and milk ejection was completely blocked (Bruckmaier et al., 1997).

If ATO was injected after manual udder preparation (ATOlate), the milk ejection was initiated by the released OT, but milk flow decreased before the udder was completely emptied. Earlier studies demonstrated that elevated OT concentrations in response to teat stimulation are already present at 30 s after the start of stimulation (Kaskous and Bruckmaier, 2011). Thus, OT could bind to the OT receptors but was competitively displaced by ATO and the milk ejection was interrupted. In agreement with earlier work, the spontaneously available milk was rather variable among individual cows between 20 to 60% of the stored milk, representing cisternal milk and a variable portion of alveolar milk (Bruckmaier et al., 1997). This may be due to differences in the exact timing of OT release and

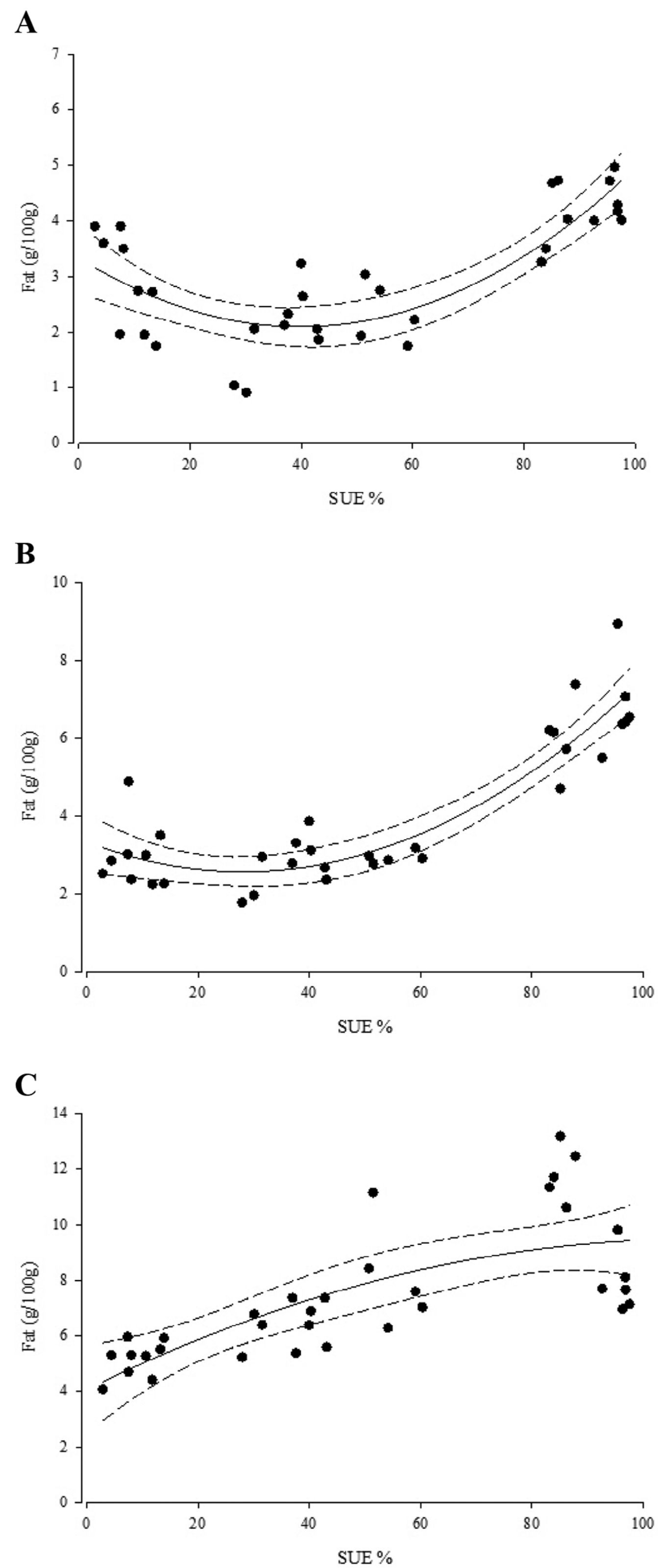

Figure 2. Fat content relative to spontaneous udder emptying (SUE) in A = milk before oxytocin (MBOT), B = strip milk (SM) and C = milk after oxytocin (MAOT). —— regression line; - - - 95% confidence band.

Table 2. Changes in milk constituents at different degrees of spontaneous udder emptying in different milk samples

Sample[2]	Parameter	No milk ejection	Partial milk ejection	Partial milk ejection	Complete milk ejection
	SUE[1]	<20%	20 - 40%	40 – 60%	>80%
MBOT					
	Fat	↓	↓	↓	↑
	Protein	↓	↑	↑↓	↓
	Lactose	↓	↑	↑	↑
	Conductivity	↑	↓	↓	↓
SM					
	Fat	↓	↓	↓	↑
	Protein	↑↓	↑	↑↓	↓
	Lactose	↓	↑	↑↓	↑↓
	Conductivity	↑	→	→	↓
MAOT					
	Fat	↓	→	→	↑
	Protein	↑	↑→	→	↓
	Lactose	↑	↑	↑	↓
	Conductivity	↓	↓	↓	↓

↓, ↑, → No common arrow indicates a significant difference ($P < 0.05$) between SUE-Groups. ↓ indicate low, ↑ indicate high and → indicate intermediate concentration of the milk constituent in the sample compared with different degrees of SUE.

[1]Spontaneous udder emptying: The milkings were classified treatment independently in SUE-Groups.

[2]Milk samples: MBOT = milk before OT; SM = strip milk; MAOT = milk after OT.

hence receptor binding during the min before the ATO injection.

Only in Control treatment the spontaneously removed milk before OT was >80% of the TMY. It represents the cisternal and alveolar milk, as far as it is ejected by physiological OT release. Only in response to the supraphysiological OT injection, residual milk of up to 20% was also removed in the control treatment which is in agreement with earlier studies of our group (Bruckmaier et al., 1994).

The similar TMY in all treatments demonstrates that the udder emptying was complete including removal of the residual milk after the used supraphysiological OT injection to overcome the OT receptor blockade by ATO (Wellnitz et al., 1999).

The variability of degree of spontaneous udder emptying in the ATO treatments allowed to classify 4 SUE-Groups. Therefore, the final evaluations of data were performed based on SUE classes instead of treatments. Unexpectedly, the ATOlate injection caused a maximum of 60% udder emptying whereas in controls the minimum udder emptying was 80%. This resulted in an unexpected gap of SUE at a range of 60 – 80%.

Changes in milk composition

The clearest differences of milk constituents at different degrees of SUE were found for the milk fat content. The fat content of SM remained almost unchanged up to 40 – 60% SUE, but was considerably higher in SM if >80% of the milk was spontaneously removed. The fat content of MBOT followed the same pattern as fat content of SM. The lack of significance among the fat content of MBOT and SM confirms that the fat content was not considerably changing within a SUE of up to 60%. This finding is in contrast to earlier studies with repeated sampling throughout milking with complete UE through endogenous OT. In these studies, the fat content of quarter milk samples increased gradually during milk removal already from the start of milk ejection until reaching highest concentration at the end of milking and in residual milk (Ontsouka et al., 2003; Sarikaya et al., 2005). An earlier interpretation of the phenomenon was a delayed fat delivery caused by the lower relative density of fat, but also capillary forces and retarded transport through the milk ducts caused by loaded membrane potential of both milk duct epithelium and fat droplets. It was detected in mice that lipid droplets were released after the OT-induced contraction of myoepithelium (Masedunskas et al., 2017). The absence of significant increase of milk fat content may be due to the cessation of myoepithelial contraction after ATO administration. Thus, the ATO administration appears to be a more suitable to study changes of milk fat contents at inhibited milk ejection due to lacking OT release than would be an interruption of milking during normal OT release. The highest milk fat content was observed in MAOT, because MAOT included the high-fat milk after overcoming the OT receptor blockade by supraphysiological OT injection.

Lactose, Na and Cl with their osmotic activity are involved in the regulation of the milk osmolarity, i.e., lower lactose levels accompany with higher electrolyte levels.

Furthermore, the EC is influenced by the fat content (Ontsouka et al., 2003; Bruckmaier et al., 2004). In the present study, the low lactose concentration in cisternal milk of MBOT and SM was accompanied with higher concentrations of Na and Cl and an elevated EC. High intramammary pressure can facilitate the transfer through the epithelial barrier which was shown by supraphysiological OT injections (Werner-Misof et al., 2007; Wall et al., 2016). A short-term reduced blood-milk barrier integrity during milking was reported due to the mechanical load of milking (Herve et al., 2017). This may be associated with opened tight junctions due to maximal alveolar contraction (Stelwagen and Singh, 2014; Wellnitz and Bruckmaier, 2021). Therefore, in the residual milk of MAOT the lactose concentration was reduced and the concentrations of Na and Cl were elevated. EC in MAOT is not influenced by the higher electrolyte concentration because the high fat content suppressed EC increase. The lowered Protein concentration at low SUE, high SUE and in residual milk of MAOT could be associated with a dilution of the protein content in these fractions, because a loss of protein due to opened tight junctions is unlikely. Higher electrolyte concentrations due to opened tight junctions could increase the water influx to keep the osmolarity of the milk in balance, and therefore the protein content would be reduced. This phenomenon could also influence the lactose concentration, i.e., its dilution through the osmotic effect of electrolytes rather than or in addition to a loss of lactose through the opened tight junctions.

Estimation of udder emptying

Clearest differences between different udder emptying were shown in the fat content of the milk, which remained almost unchanged up to 60% SUE. Therefore, the fat content in spontaneously removed milk or in a strip milk sample is suitable to differentiate between complete and incomplete udder emptying. Incomplete udder emptying could be the result of totally lack of OT with complete inhibited milk ejection or reduced OT release with interrupted milk ejection (Bruckmaier et al., 1997). Other milk constituents could help to differentiate between these 2 situations, such as EC and lactose in spontaneous removed milk and EC in strip milk. In most milking systems the spontaneously removed milk is directly transported in the milking line system. Thus, the spontaneously obtained milk is not easily available. Under practical conditions, a strip milk sample collected after cluster detachment is the most practical sampling method, even at an individual quarter level if required. Therefore, with the fat content and the EC in strip milk the udder emptying could be estimated into complete, partial or incomplete (only cisternal milk is removable, respectively). The EC can be easily measured on farm, whereas fat is measured in a laboratory. Thus, EC allows a fast interpretation of udder emptying on farm and fat can be added up later. Our results demonstrated that the additional consideration of foremilk concentration of individual milk constituents did not show any advantage to estimate the degree of udder emptying based on the milk fat content (data not shown). It has to be taken into account that the results of our study are based on 10 Holstein dairy cows and absolute values may vary between individual cows, breeds, or different milking procedures and measurements must always be referred to the corresponding herd.

CONCLUSION

The blockade of the OT-receptor with ATO by different injection time points is suitable to simulate milk ejection disorders and to induce different degrees of incomplete udder emptying. The complete or partial blockade of the OT effect on the mammary gland in response to tactile udder stimulation affects primarily the fat content in spontaneous removed milk and in strip milk samples. The absence of an increased fat content up to 60% SUE in the ATO treatments confirms the OT-induced fat secretion during milking of cows. Therefore, ATO-induced lacking or incomplete milk ejection is likely a more suitable simulation of spontaneous inhibition of milk ejection than incomplete milk removal by pre-term detachment of the milking cluster during milking with normal OT release. The results show that a low bulk milk fat content in cow-calf contact systems can be an indicator of disturbed milk ejection. Based on the fat content and the EC in strip milk samples collected after cluster detachment the udder emptying can be estimated as complete or incomplete which allows a reliable detection of milk ejection disorders.

ACKNOWLEDGMENTS

We are grateful to Mrs Chantal Philipona and Mrs Nathalie Besuchet, both Veterinary Physiology, for expert laboratory work. The study was financially supported be DeLaval, Tumba, Sweden. The authors have not stated any conflicts of interest.

REFERENCES

Barth, K. 2020. Effects of suckling on milk yield and milk composition of dairy cows in cow-calf contact systems. J. Dairy Res. 87(S1):133–137. https://doi.org/10.1017/S0022029920000515.

Belo, C. J., and R. M. Bruckmaier. 2010. Suitability of low-dosage oxytocin treatment to induce milk ejection in dairy cows. J. Dairy Sci. 93:63–69. https://doi.org/10.3168/jds.2009-2084.

Belo, C. J., S. Schlegel, J. Moll, E. Möstl, and R. M. Bruckmaier. 2009. Milk ejection disorders in Swiss dairy cows: a field study. J. Dairy Res. 76:222–228. https://doi.org/10.1017/S002202990900394X.

Bruckmaier, R. M., and J. W. Blum. 1998. Oxytocin release and milk removal in ruminants. J. Dairy Sci. 81:939–949. https://doi.org/10.3168/jds.S0022-0302(98)75654-1.

Bruckmaier, R. M., C. E. Ontsouka, and J. W. Blum. 2004. Fractionized milk composition in dairy cows with subclinical mastitis. Vet. Med. (Praha) 49:283–290. https://doi.org/10.17221/5706-VETMED.

Bruckmaier, R. M., D. Schams, and J. W. Blum. 1994. Continuously elevated concentrations of oxytocin during milking are necessary for complete milk removal in dairy cows. J. Dairy Res. 61:323–334. https://doi.org/10.1017/S0022029900030740.

Bruckmaier, R. M., O. Wellnitz, and J. W. Blum. 1997. Inhibition of milk ejection in cows by oxytocin receptor blockade, α-adrenergic receptor stimulation and in unfamiliar surroundings. J. Dairy Res. 64:315–325. https://doi.org/10.1017/S002202999700232X.

Herve, L., H. Quesnel, V. Lollivier, J. Portanguen, R. M. Bruckmaier, and M. Boutinaud. 2017. Mammary epithelium disruption and mammary epithelial cell exfoliation during milking in dairy cows. J. Dairy Sci. 100:9824–9834. https://doi.org/10.3168/jds.2017-13166.

Kaskous, S., and R. M. Bruckmaier. 2011. Best combination of pre-stimulation and latency period duration before cluster attachment for efficient oxytocin release and milk ejection in cows with low to high udder-filling levels. J. Dairy Res. 78:97–104. https://doi.org/10.1017/S0022029910000816.

Kaskous, S. H., D. Weiss, Y. Massri, A.-M. B. Al-Daker, A.-D. Nouh, and R. M. Bruckmaier. 2006. Oxytocin release and lactation performance in Syrian Shami cattle milked with and without suckling. J. Dairy Res. 73:28–32. https://doi.org/10.1017/S0022029905001329.

Masedunskas, A., Y. Chen, R. Stussman, R. Weigert, and I. H. Mather. 2017. Kinetics of milk lipid droplet transport, growth, and secretion revealed by intravital imaging: lipid droplet release is intermittently stimulated by oxytocin. Mol. Biol. Cell 28:935–946. https://doi.org/10.1091/mbc.e16-11-0776.

Ontsouka, C. E., R. M. Bruckmaier, and J. W. Blum. 2003. Fractionized milk composition during removal of colostrum and mature milk. J. Dairy Sci. 86:2005–2011. https://doi.org/10.3168/jds.S0022-0302(03)73789-8.

Sarikaya, H., C. Werner-Misof, M. Atzkern, and R. M. Bruckmaier. 2005. Distribution of leucocyte populations, and milk composition, in milk fractions of healthy quarters in dairy cows. J. Dairy Res. 72:486–492. https://doi.org/10.1017/S0022029905001317.

Stelwagen, K., and K. Singh. 2014. The role of tight junctions in the mammary gland. J. Mammary Gland Biol. Neoplasia 19:131–138. https://doi.org/10.1007/s10911-013-9309-1.

Tančin, V., L. Harcek, J. Broucek, M. Uhrincat, and S. Mihina. 1995. Effect of suckling during early lactation and changeover to machine milking on plasma oxytocin and cortisol levels and milking characteristics in Holstein cows. J. Dairy Res. 62:249–256. https://doi.org/10.1017/S0022029900030958.

Wall, S. K., O. Wellnitz, L. E. Hernández-Castellano, A. Ahmadpour, and R. M. Bruckmaier. 2016. Supraphysiological oxytocin increases the transfer of immunoglobulins and other blood components to milk during lipopolysaccharide and lipoteichoic acid induced mastitis in dairy cows. J. Dairy Sci. 99:9165–9173. https://doi.org/10.3168/jds.2016-11548.

Wellnitz, O., and R. M. Bruckmaier. 2021. Invited Review: The role of the blood-milk barrier and its manipulation for the efficacy of the mammary immune response and milk production. J. Dairy Sci. 104:6376–6388. https://doi.org/10.3168/jds.2020-20029.

Wellnitz, O., R. M. Bruckmaier, C. Albrecht, and J. W. Blum. 1999. Atosiban, an oxytocin receptor blocking agent: pharmacokinetics and inhibition of milk ejection in dairy cows. J. Dairy Res. 66:1–8. https://doi.org/10.1017/S0022029998003227.

Werner-Misof, C., M. W. Pfaffl, H. H. D. Meyer, and R. M. Bruckmaier. 2007. The effect of chronic oxytocin-treatment on the bovine mammary gland immune system. Vet. Med. (Praha) 52:475–486. https://doi.org/10.17221/2059-VETMED.

Zipp, K. A., K. Barth, E. Rommelfanger, and U. Knierim. 2018. Responses of dams versus non-nursing cows to machine milking in terms of milk performance, behaviour and heart rate with and without additional acoustic, olfactory or manual stimulation. Appl. Anim. Behav. Sci. 204:10–17. https://doi.org/10.1016/j.applanim.2018.05.002.

ORCIDS

Benjamin Jenni https://orcid.org/0009-0005-7560-6386
Olga Wellnitz https://orcid.org/0000-0002-5817-3428
Rupert M. Bruckmaier https://orcid.org/0000-0002-9374-5890