Milk ejection in dairy cows at different degrees of udder filling

RUPERT M. BRUCKMAIER* AND MONIKA HILGER

Institut für Physiologie, Technische Universität München, Weihenstephaner Berg 3, D-85350 Freising, Germany

(*Received 31 July 2000 and accepted for publication 14 February 2001*)

SUMMARY. Occurrence of milk ejection and course of milk removal were investigated in 18 dairy cows at milking intervals of 4, 8 and 12 h in early, mid or late lactation. Milk ejection occurred fastest in early lactation at a milking interval of 12 h and was delayed at short milking intervals and in late lactation. Storage capacity of the udder was estimated and the actual milk yields of experimental milkings were calculated as a percentage of storage capacity, i.e. degree of udder filling. It was shown that the occurrence of milk ejection after the start of teat stimulation is a function of udder filling. The relationship between the degree of udder filling and the delay from the start of milking until commencement of milk ejection followed a linear regression curve. Changes in occurrence and course of milk ejection have to be considered in practical milking, mainly in late stages of lactation and after short milking intervals. In automatic milking systems where variable and sometimes extremely short milking intervals occur, the duration of pre-milking udder preparation should be adapted to the expected milk yield at each individual milking procedure.

KEYWORDS: Cow, oxytocin, milk ejection, milking interval, udder filling.

Less than 20% of the milk stored in a cow's mammary gland is usually present in the cisternal cavities and large milk ducts and immediately available for milk removal after milking intervals of 10–14 h (Knight *et al*. 1994; Pfeilsticker *et al*. 1996; Davis *et al*. 1998). After a shorter interval from the previous milking the cisternal fraction is even smaller and can be close to zero (Knight *et al*. 1994). The major milk fraction, the alveolar milk, only becomes available by milk ejection (Schams *et al*. 1984; Bruckmaier *et al*. 1994). Tactile udder stimulation causes release of oxytocin (OT) from the posterior pituitary. Myoepithelial contraction and ejection of milk into the cisternal cavities only commences in response to elevated OT blood concentrations (Crowley & Armstrong, 1992; Bruckmaier & Blum, 1998). The release of OT in response to teat stimulation remains unchanged or increases during the course of lactation, whereas milk ejection, i.e. the response to OT release, is delayed in late lactation when milk yields decrease (Mayer *et al*. 1991). On the other hand, the onset and course of milk ejection after the start of a tactile teat stimulation is not different between high and low yielding cows if the animals are in a similar stage of lactation (Wellnitz *et al*. 1999). To test the hypothesis that the occurrence of milk ejection

* For correspondence; e-mail: bruckmaier@weihenstephan.de

depends on the amount of milk stored in the udder, not with regard to its absolute amount, but as a function of maximum storage capacity of the gland, we investigated the occurrence of milk ejection in early, mid and late lactational stages at milking intervals of 4, 8 and 12 h.

MATERIALS AND METHODS

Animals

Eighteen healthy experimental cows of the Holstein Friesian (12) and German Braunvieh X Brown Swiss (6) breeds were used from two experimental farms. Animals were in their first to fifth lactation and were designated to three lactational stages with six cows (4 Holstein, 2 Braunvieh) in each: early (weeks 3–11), mid (weeks 22–31) and late (weeks 34–43) lactation.

Experimental design

Routine milkings were performed at 05.00 and at 16.00 on both farms. All experiments were performed during afternoon milking time 4, 8, or 12 h since the previous milking. For measurements at the 12-h interval, experiments were performed exactly 12 h after morning milking. To achieve 8-h and 4-h milking intervals, cows were additionally milked at 08.00 and 12.00, to give these respective milking intervals during afternoon milking. The exact starting time of milking for each individual cow was recorded and experiments were performed on the minute 4 h or 8 h later in each animal. To avoid influences of experimental days upon the treatments, two cows from each group were milked at 4, 8 or 12 h intervals on each day in random sequence.

Milk ejection and milk removal were studied according to three different experimental protocols. One experiment per day was performed. The pattern of intramammary pressure (IMP) in the teat cistern was recorded during manual teat stimulation until milk ejection occurred immediately before the start of milking (protocol 1). Milk flow curves were recorded during milking with (protocol 2) or without (protocol 3) a 1-min manual prestimulation.

Measurements of intramammary pressure, milk flow and oxytocin

IMP was measured through a cannula in the teat channel by using a strain gauge system as previously described (Bruckmaier *et al*. 1994). Parameters evaluated were baseline pressure (pressure before teat stimulation), ejection pressure (pressure maximum in response to teat stimulation), induction time (time from start of stimulation until start of pressure increase), and ejection time (time from start of stimulation until ejection pressure was reached). Milk flow was continuously recorded by a mobile system (Lactocorder) as described previously (Bruckmaier & Blum, 1996). The occurrence of alveolar milk ejection was visible as the second milk flow increment in bimodal milk flow curves. Although the flows of cisternal and alveolar milk fractions are often contiguous it was previously shown that the nadir of the bimodal milk flow curve represents the commencement of alveolar milk ejection into the cistern (Bruckmaier & Blum, 1996).

In six animals, two of each lactational stage, blood samples were taken via a catheter in the jugular vein during IMP recordings at -1, 0, 1, 2, 3 min before and during teat stimulation (0 = start of stimulation) at 4, 8 and 12 h after the preceding milking. Blood samples were treated with EDTA to prevent coagulation and centrifuged at 1500 ***g*** for 15 min immediately after each experiment. Blood plasma was stored at −20 °C until used for radioimmunological determination of OT concentration according to Schams (1983).

Mathematical calculations and statistical evaluations

Data are presented as means ± standard error of means (SEM). Degree of udder filling was calculated as actual milk yield (percentage of maximum storage capacity). Maximum storage capacity of the mammary gland was estimated as half the daily milk yield in month 2 of the respective lactation, assuming that all experimental animals were routinely milked twice daily. For statistical evaluation, analysis of variance was calculated based on least-square means using the GLM procedure of SAS (SAS 6.11). Influences of animal, farm, milking interval, and stage of lactation or udder filling class were tested. The Least-Significant-Difference (LSD) test was used to distinguish between treatment means.

RESULTS

Oxytocin

Baseline OT concentrations were 4·2 ± 0·5, 1·8 ± 0·3 and 4·6 ± 0·8 pg/ml at milking intervals of 4, 8 and 12 h, respectively. Within 1 min after the start of manual teat stimulation, OT concentrations increased and AUC/min during 3 min of stimulation was 28·4 ± 15·6, 13·7 ± 3·1 and 61·5 ± 23·0 pg/ml at 4-, 8- and 12-h intervals respectively. Both baseline values and values during stimulation were significantly lower at 8 h than at 12 h ($P < 0{\cdot}05$), while the means at 4 h did not significantly differ from the others.

Intramammary pressure

Means of IMP traits at different stages of lactation and at different milking intervals are shown in Table 1. Baseline pressure and ejection pressure were highest in early lactation at the 12-h interval and lowest in late lactation at the 4-h interval. Within each stage of lactation, IMP values increased with increasing milking interval. Within similar milking intervals its values decreased towards late lactation, except for the baseline pressure at the 4-h interval and ejection pressure at the 8-h interval, where the numerically highest baseline pressure was measured at mid-lactation. Induction time and ejection time were numerically, but not significantly shortest in early lactation at the 12-h interval. Induction time was longest in late lactation at the 4-h interval while the longest ejection time was found at the 4-h interval in early lactation. Within each lactational stage, both induction time and ejection time decreased with increasing milking interval.

IMP traits, classified by degree of udder filling estimated by the milk yield obtained during milking after IMP measurements, are shown in Table 2. Baseline and ejection pressure increased continuously with increasing degree of udder filling. In contrast, induction and ejection times had their highest values at the lowest degree of filling and diminished continuously with increasing degree of udder filling.

Milk yield and milk flow

Milk yields (Table 3) were reduced with stage of lactation and increased with increasing milking intervals in each lactational stage. Corresponding milk yields with and without prestimulation were not significantly different except for milk yields in

Table 1. *Intramammary pressure at different milking intervals in different stages of lactation*

Parameter	Unit	Milking interval (h)	Weeks 3–11	Weeks 22–31	Weeks 34–43
Baseline pressure	kPa	4	$1{\cdot}2 \pm 0{\cdot}2^{C}$	$1{\cdot}6 \pm 0{\cdot}3^{B}$	$1{\cdot}1 \pm 0{\cdot}2^{B}$
		8	$1{\cdot}9 \pm 0{\cdot}3^{B}$	$1{\cdot}7 \pm 0{\cdot}4^{B}$	$1{\cdot}4 \pm 0{\cdot}2^{AB}$
		12	$3{\cdot}0 \pm 0{\cdot}2^{Aa}$	$2{\cdot}5 \pm 0{\cdot}4^{Aa}$	$1{\cdot}7 \pm 0{\cdot}2^{Ab}$
Ejection pressure	kPa	4	$3{\cdot}6 \pm 0{\cdot}2^{Ca}$	$3{\cdot}6 \pm 0{\cdot}4^{Ba}$	$2{\cdot}9 \pm 0{\cdot}2^{Bb}$
		8	$4{\cdot}5 \pm 0{\cdot}3^{Ba}$	$4{\cdot}6 \pm 0{\cdot}4^{Aa}$	$3{\cdot}4 \pm 0{\cdot}4^{Bb}$
		12	$5{\cdot}3 \pm 0{\cdot}3^{Aa}$	$5{\cdot}0 \pm 0{\cdot}4^{Aa}$	$4{\cdot}2 \pm 0{\cdot}4^{Ab}$
Induction time	s	4	92 ± 6^{A}	95 ± 8^{A}	96 ± 9^{A}
		8	79 ± 7^{B}	83 ± 8^{AB}	78 ± 5^{B}
		12	64 ± 5^{C}	72 ± 5^{B}	65 ± 3^{C}
Ejection time	s	4	150 ± 13^{A}	144 ± 14^{A}	145 ± 15^{A}
		8	132 ± 11^{A}	118 ± 10^{AB}	140 ± 6^{A}
		12	102 ± 10^{B}	108 ± 8^{B}	114 ± 3^{B}

^{A,B,C} Means without common superscript letter within column are significantly different ($P < 0{\cdot}05$).
^{a,b} Means without common superscript letter within line are significantly different ($P < 0{\cdot}05$).

Table 2. *Intramammary pressure at different degrees of udder filling*

		Filling class				
Parameter	Unit	0–20 %	20·1–40 %	40·1–60 %	60·1–80 %	80·1–100 %
Number of observations		6	18	14	13	3
Milk yield	kg	$3{\cdot}1 \pm 0{\cdot}4^{e}$	$6{\cdot}4 \pm 0{\cdot}4^{d}$	$10{\cdot}4 \pm 0{\cdot}4^{c}$	$15{\cdot}5 \pm 1{\cdot}1^{b}$	$17{\cdot}5 \pm 1{\cdot}7^{a}$
Mean udder filling	%	$14{\cdot}1 \pm 1{\cdot}6^{e}$	$28{\cdot}5 \pm 1{\cdot}2^{d}$	$50{\cdot}4 \pm 1{\cdot}5^{c}$	$68{\cdot}0 \pm 2{\cdot}8^{b}$	$84{\cdot}6 \pm 1{\cdot}1^{a}$
Baseline pressure	kPa	$1{\cdot}0 \pm 0{\cdot}1^{c}$	$1{\cdot}4 \pm 0{\cdot}1^{c}$	$1{\cdot}8 \pm 0{\cdot}2^{b}$	$2{\cdot}8 \pm 0{\cdot}2^{a}$	$3{\cdot}3 \pm 0{\cdot}3^{a}$
Ejection pressure	kPa	$2{\cdot}7 \pm 0{\cdot}2^{d}$	$3{\cdot}8 \pm 0{\cdot}2^{c}$	$4{\cdot}3 \pm 0{\cdot}2^{b}$	$5{\cdot}2 \pm 0{\cdot}2^{b}$	$5{\cdot}5 \pm 0{\cdot}5^{a}$
Induction time	s	80 ± 8^{b}	89 ± 4^{a}	79 ± 4^{b}	66 ± 3^{bc}	56 ± 1^{c}
Ejection time	s	139 ± 10^{a}	135 ± 6^{a}	133 ± 6^{a}	100 ± 5^{b}	99 ± 27^{b}

^{a,b,c,d,e} Means without common superscript letter within line are significantly different ($P < 0{\cdot}05$).

Table 3. *Milk yields at different milking intervals in different stages of lactation without and with prestimulation and delay until start of milk ejection during milking without prestimulation*

Parameter	Unit	Prestimulation	Milking interval (h)	Weeks 3–11	Weeks 22–31	Weeks 34–43
Milk yield	kg	Without	4	$7{\cdot}9 \pm 0{\cdot}8^{Ca}$	$5{\cdot}1 \pm 1{\cdot}0^{Cb}$	$3{\cdot}4 \pm 0{\cdot}7^{Bc}$
			8	$14{\cdot}4 \pm 1{\cdot}2^{Ba}$	$10{\cdot}4 \pm 1{\cdot}5^{Bb}$	$7{\cdot}5 \pm 1{\cdot}4^{Ac}$
			12	$19{\cdot}1 \pm 1{\cdot}0^{Aa}$	$13{\cdot}1 \pm 1{\cdot}5^{Ab}$	$8{\cdot}7 \pm 1{\cdot}4^{Ac}$
Milk yield	kg	With	4	$6{\cdot}6 \pm 1{\cdot}1^{Ca}$	$5{\cdot}8 \pm 1{\cdot}1^{Cab}$	$4{\cdot}7 \pm 1{\cdot}1^{Bb}$
			8	$14{\cdot}2 \pm 1{\cdot}5^{Ba}$	$9{\cdot}5 \pm 1{\cdot}5^{Bb}$	$7{\cdot}0 \pm 1{\cdot}3^{Ac}$
			12	$18{\cdot}2 \pm 1{\cdot}6^{Aa}$	$12{\cdot}3 \pm 1{\cdot}5^{Ab}$	$8{\cdot}5 \pm 1{\cdot}2^{Ac}$
Delay until start of milk ejection	s	Without	4	73 ± 4^{Ac}	107 ± 11^{Aa}	91 ± 9^{Ab}
			8	58 ± 5^{ABa}	74 ± 5^{Bab}	73 ± 6^{Bb}
			12	50 ± 5^{Bb}	63 ± 4^{Ba}	71 ± 4^{Ba}

^{A,B,C} Means without common superscript letter within column are significantly different ($P < 0{\cdot}05$).
^{a,b,c} Means without common superscript letter within line are significantly different ($P < 0{\cdot}05$).

late lactation at the 4-h interval, where milk yield was significantly lower without prestimulation.

Time until removal of the first alveolar milk (Table 3, Fig. 1) was only measurable during milking without prestimulation because the milk flow curves showed their

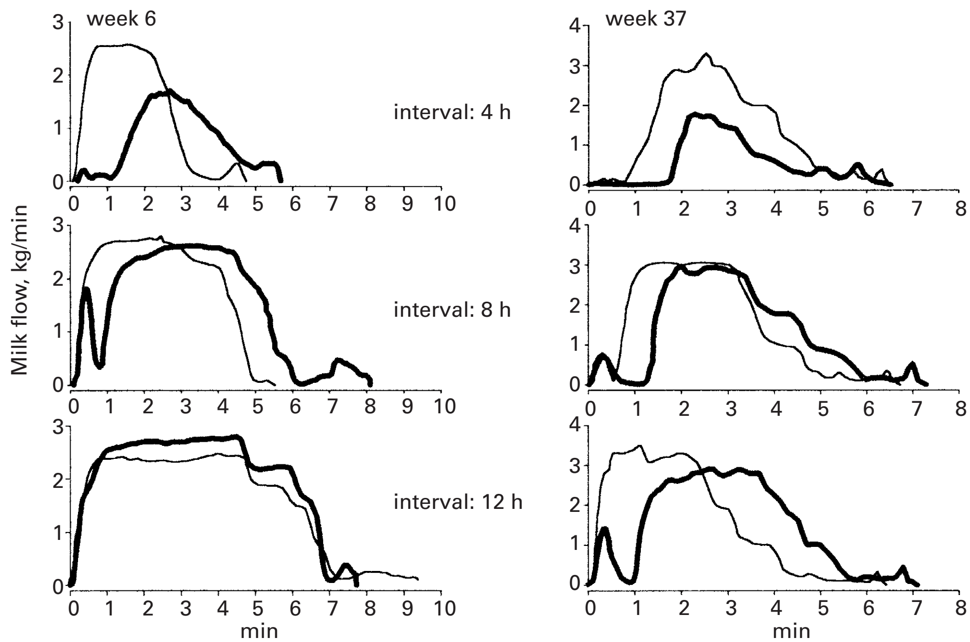

Fig. 1. Milk flow curves of two respresentative animals, one in week 6 and one in week 37 of lactation, at milking intervals of 4, 8 and 12 h respectively. Milkings were performed with a 1-min manual prestimulation (*thin lines*) or without prestimulation (*thick lines*). 0 min = start of milking.

Table 4. *Delay until alveolar milk ejection at different degrees of udder filling*

		Filling class				
Parameter	Unit	0–20 %	20·1–40 %	40·1–60 %	60·1–80 %	80·1–100 %
Number of observations		7	19	13	10	5
Mean udder filling	%	12±2	30±1	53±2	69±2	91±2
Delay until start of milk ejection	s	107±9[a]	78±3[b]	68±4[c]	59±2[c]	47±4[c]

[a,b,c] Means without common superscript letter within line are significantly different ($P < 0.05$).

characteristic bimodal shape where the nadir corresponds with the commencing milk ejection. AUC of the first peak in bimodal milk flow curves represents the cisternal fraction. AUC was not mathematically evaluated because in many cases the interruption of milk flow was not complete and therefore the cisternal fraction was not quantitatively defined. However, Fig. 1 demonstrates that the cisternal fraction was smallest after short milking intervals in late lactation and largest after long milking intervals in early lactation.

The delay from start of milking until start of milk ejection (Table 3) at milkings without prestimulation decreased with increasing milking interval in all lactational stages. It was numerically shortest in early and longest in mid lactation. Start of milk ejection was clearly a function of degree of udder filling (Table 4, Fig. 2). Divided into filling classes (Table 4), the time until start of milk ejection was longest in the class 0–20 % and decreased gradually with class until class 80–100 %, where the mean value was less than half of class 0–20 %. As shown in Fig. 2, the relationship between the degree of udder filling and start of milk ejection followed a linear regression

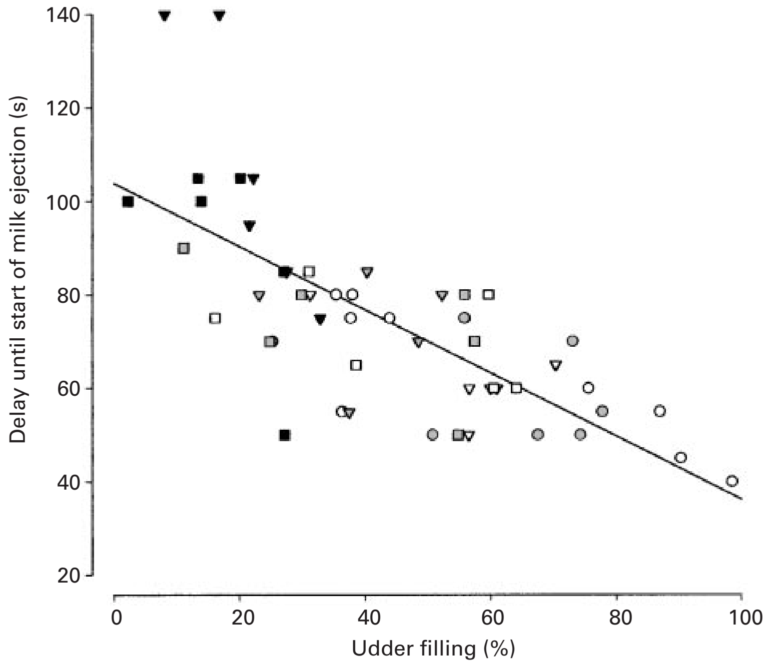

Fig. 2. Relationship between degree and udder filling and delay until start of milk ejection. *Circles* early lactation, *triangles* mid lactation, *rectangles* late lactation, *filled symbols* 4-h interval, *crossed symbols* 8-h interval and *plain symbols* 12-h interval.

[$y = 103{\cdot}68 - 0{\cdot}68x$; x = degree of udder filling (%), y = start of milk ejection (s)]. Pearson's coefficient of correlation was $r = 0{\cdot}73$ ($P < 0{\cdot}05$).

DISCUSSION

Our results demonstrate changes of commencement and course of milk ejection due to variation of lactational stage and milking interval. This may principally be due to changes in the level of OT release or on the level of response to released OT. We have previously shown that milking-related OT release increases, rather than decreases, during the course of lactation (Mayer *et al.* 1991). In the present study, baseline concentrations and release of OT in response to teat stimulation were different between milking intervals with lowest values at the 8-h interval and highest values at the 12-h interval. However, OT concentrations were elevated after 1 min teat stimulation at all milking intervals. Although more OT was released at the 4-h interval than at 8-h interval, the period from the start of stimulation until commencement of milk ejection was much longer at the 4-h than 8-h interval. It has been previously shown that the amount of released OT is usually variable and that surmounting a threshold of OT is sufficient to induce maximum milk ejection (Schams *et al.* 1984; Bruckmaier *et al.* 1994). A highly repeatable course of milk ejection despite a considerable variation of OT release at individual milkings was observed in several investigations (Schams *et al.* 1984; Mayer *et al.* 1991). In contrast, the timing of the OT increase is crucial for the course of milk ejection (Bruckmaier *et al.* 1994; Bruckmaier & Blum, 1996). The variation of absolute OT concentrations at different milking intervals is probably due to the relatively small number of animals investigated for OT. It can be assumed that the release of OT was not a limiting factor for the occurrence of milk ejection in our experiments.

Induction and course of milk ejection were delayed after short milking intervals and in late lactation. Related to an estimated degree of udder filling, this delay was clearly due to reduced filling of the mammary gland. It can be assumed that the number and size of alveoli in the mammary gland is not changing due to short-term variation of the milking interval, and only slight changes during the course of lactation are expected. Despite different degrees of filling with milk, the surface of the alveoli therefore remains similar. The surface of the alveoli is covered with myoepithelial cells which contract in response to OT. The less these alveoli are filled with milk the more contraction is necessary to cause an efficient transfer of milk via ductal pathways into the cistern. It is obvious that greater contraction of the myoepithelial cells and transfer of small amounts of alveolar milk through the duct system takes more time. Thus the delayed milk ejection during late lactation (Mayer *et al*. 1991), sometimes interpreted as an increased requirement for prestimulation, has the same reason as the delayed milk ejection after short milking intervals, i.e. decreased udder filling. The time until start of milk ejection during milking without prestimulation was longest in the class with the lowest degree of udder filling. This finding is supported by earlier results where partial milk ejections caused fractionised removal of alveolar milk and where the response time of milk ejection to injected OT was prolonged with each portion of milk removed (Bruckmaier *et al*. 1994). During IMP measurements, the longest induction time of milk ejection was measured in the class 20–40% of filling, whereas the means of class 0–20% was numerically shorter. It has to be considered that even in late lactation and at the 4-h interval many animals produced more than 20% of maximal milk. Only six measurements could be included in the class 0–20%.

The time until start of milk ejection in milkings without prestimulation and induction time of milk ejection during IMP measurements should have been similar (Bruckmaier & Blum, 1996). In this study, the mean induction time was mostly slightly longer than the start of milk ejection during milking. IMP and milk flow were recorded on different experimental days and the degree of udder filling was not exactly the same. Furthermore, IMP only reflects milk ejection in one front quarter whereas the start of milk ejection during milking reflects milk ejection in the quarter where it occurs at first. Possibly, milk ejection in front quarters occurs slightly later than in the hind quarters.

As expected, milk yields were lower in late lactation and after short milking intervals than in early lactation and after long milking intervals. Milk yield was not different with or without prestimulation, except for the 4-h interval in late lactation. This shows the importance of a sufficient prestimulation at low degrees of udder filling, where the small or missing cisternal milk fraction (Knight *et al*. 1994) comes along with extremely late commencement of milk ejection. As a consequence, milking empty teats cannot be avoided during early milking until milk ejection occurs.

Implications for practical milking

These data clearly show the effect of udder filling on commencement and course of milk ejection. This relationship has to be considered in traditional milk systems during late lactational stages. In addition, delayed milk ejection in little-filled udders is crucial in automatic milking systems (AMS) where cows enter voluntarily for milking. Extremely short milking intervals can occur, if the access of cows is not set to a minimum threshold. In AMS, a specific prestimulation at each milking, automatically calculated from the expected milk yield at a given lactational stage and milking interval could help to provide optimal udder preparation.

We thank Mrs. C. Fochtmann, Ms. J. Macuhova and Mr. S. Wittmann for their help during the experiments and the farms Thalhausen and Veitshof, Weihenstephan, for providing the experimental animals. This study was financially supported by DeLaval, Glinde, Germany.

REFERENCES

Bruckmaier, R. M. & Blum, J. W. 1996 Simultaneous recording of oxytocin release, milk ejection and milk flow during milking of dairy cows with and without prestimulation. *Journal of Dairy Research* **63** 201–208

Bruckmaier, R. M. & Blum, J. W. 1998 Oxytocin release and milk removal in ruminants. *Journal of Dairy Science* **81** 939–949

Bruckmaier, R. M., Schams, D. & Blum, J. W. 1994 Continuously elevated concentrations of oxytocin during milking are necessary for complete milk removal in dairy cows. *Journal of Dairy Research* **61** 323–334

Crowley, W. R. & Armstrong, W. E. 1992 Neurochemical regulation of oxytocin secretion in lactation. *Endocrine Reviews* **13** 33–65

Davis, S. R., Farr, V. C., Copeman, P. J. A., Carruthers, V. R., Knight, C. H. & Stelwagen, K. 1998 Partitioning of milk accumulation between cisternal and alveolar compartments of the bovine udder: relationship to production loss during once daily milking. *Journal of Dairy Research* **65** 1–8

Knight, C. H., Hirst, D. & Dewhurst, R. J. 1994 Milk accumulation and distribution in the bovine udder during the interval between milkings. *Journal of Dairy Research* **61** 167–177

Mayer, H., Bruckmaier, R. M. & Schams, D. 1991 Lactational changes in oxytocin release, intramammary pressure and milking characteristics in dairy cows. *Journal of Dairy Research* **58** 159–169

Pfeilsticker, H.-U., Bruckmaier, R. M. & Blum, J. W. 1996 Cisternal milk in the dairy cow during lactation and after preceding teat stimulation. *Journal of Dairy Research* **63** 509–515

SAS 1995 SAS/STAT User's Guide, release 6.11. SAS Institute Inc., Cary, NC, USA

Schams, D. 1983 Oxytocin determination by radioimmunoassay. III. Improvement to subpicogram sensitivity and application to blood levels in cyclic cattle. *Acta Endocrinologica* **103** 180–183

Schams, D., Mayer, H., Prokopp, A. & Worstorff, H. 1984 Oxytocin secretion during milking in dairy cows with regard to the variation and importance of a threshold level for milk removal. *Journal of Endocrinology* **102** 337–343

Wellnitz, O., Bruckmaier, R. M. & Blum, J. W. 1999 Milk ejection and milk removal of single quarters in high yielding dairy cows. *Milchwissenschaft* **54** 303–306