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Studies on milk ejection and milk removal during machine milking in different species

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Anamariji i Mihovilu

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ABBREVIATIONS

AMS	automatic milking system
B-mode	brightness-mode
EDTA	ethylenediaminetetraacetic acid
IMP	intramammary pressure
OT	oxytocin
PVN	paraventricular nucleus
RIA	radioimmunoassay
SON	supraoptic nucleus

1. INTRODUCTION

1.1. Mammary gland anatomy

The mammary gland is a skin gland common to all mammals. Its function is to nourish and protect the neonate. The mammary gland is a milk-producing gland in female mammals and present in a rudimentary and non-functional form in males of most species. The mammary gland of eutherian species can be simple or complex. A simple gland is drained through a single orifice at a surface, while a complex gland has more orifices and each draining a functionally separated single gland. The size and shape is species dependent (Schmidt, 1971). Several species have a series of glands, while others have groups of two or four glands that form an udder. Major components of the mammary gland are: a secretory system, a ductular system and teats.

The udder of the cow consists of four separate glands. It is located in the inguinal region and attached to ventral body wall of the cow. The udder is covered with hair, except for the teats. The mammary gland is drained through one teat (Figure 1) which has one orifice. Supernumerary teats with or without a small orifices or with connections to one of the normal mammary glands could occur in some cows (Turner, 1952). The intramammary groove divides the left and the right halves of the udder. Fore and rear quarters are disjoined by a thin connective tissue septa. Fore teats are usually longer than rear teats. The milk production in rear and fore quarters is approximately 60 and 40%, respectively. The teat length in Holstein cows is 4 to 7 cm with a diameter of 2.2 to 3.0 cm (Rogers and Spencer, 1991). The Furstenberg's rosette is located between the teat canal and the teat cistern. The teat canal is the only connection between the mammary gland and the outside environment. It is closed between milkings to impede leakage. The length of the teat canal usually varies between 8 and 12 mm. It increases in length and diameter with proceeding lactation number (Bramley et al., 1992). It serves as a main barrier against infection. The teat canal is lined with keratin, a material derived from epidermal cells which consists of fatty acids which have bacteriostatic or bactericidal properties (Hogan et al., 1987). The keratin closes the teat canal, except during milking time. Throughout milking, a substantial loss of keratin occurs by the shear force induced during milk removal and by dissolving of the certain keratin components (Bitman et al., 1991).

The gland or udder cistern stores cisternal milk between milkings. Usually large milk ducts are draining milk from the secretory tissue into the cisternal cavities. The gland cistern is variable in size but usually stores around 100 to 400 ml of milk (Hurley, 2002) or is approximately the size of an orange with a storage average of 200 ml milk (Akers, 2002). The udder suspensory system should be strong to keep up the proper attachment of the udder to the body of the cow. The median and lateral suspensory ligaments provide the main support for the udder. The median suspensory ligaments are attached to the strong tendons of the abdominal muscles and to the pelvic bone (Emmerson, 1941). The mammary gland interior is made up of connective (fibrous and adipose tissue) and secretory tissue. The lobes consist of groups of lobules which are surrounded by a connective tissue cover. The lobules are clusters of alveoli which are separated from other clusters by fibrous connective tissue. An alveolus consists of a single layer of secretory epithelial cells attached to a basal membrane, a vascular system and myoepithelial cells. The secretory epithelial cells are surrounded by myoepithelial cells. Myoepithelial cells which are under hormonal control have characteristics of the smooth muscle and they are able to contract. An alveolus with myoepithelial cells is wrapped into the network of the blood capillaries and lymph vessels. The alveoli produce milk constituents from blood precursors.

The udder of the ewe is located in the inguinal region and consists of two mammary glands, each drained by a teat (Figure 1) with a single teat canal. The skin of the teat is sparsely covered with fine hair. The teats are cone-shaped with a length of 1 to 3 cm (Wendt et al., 1994). Supernumerary teats are quite common. Similar as in sheep, the udder of the goat consists of two halves, each with a single mammary gland drained through a single teat (Figure 1). The goat teats and udder are generally larger than that of sheep. It consists of several folds of mucous membranes, each having secondary folds. The mammary gland of the mare is composed of two halves, each of them consisting of two gland complexes with two cisterns and one teat (Figure 1) with two orifices. The glands are separated by the septum along the prominent intramammary groove. Each mammary gland consists of a mammary portion and a teat, while the glandular portions have 2 or sometimes 3 lobes (Chavatte, 1997). There is one orifice for each lobe in the corresponding teat.

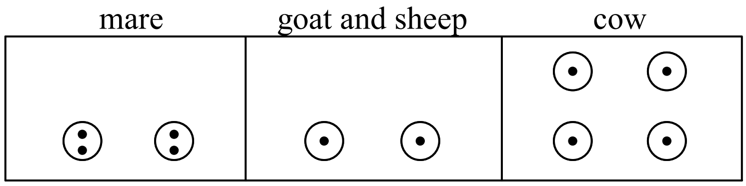

Figure 1. Teat orifices situated in the inguinal region in horse, goat, sheep and cattle. Circles indicate mammary gland and black points indicate teat orifices

1.2. Distribution of milk fractions before milk ejection

The epithelial cells secrete milk between milkings, which is removed from the gland during milking. There are two milk fractions present in the udder before the start of milking: cisternal and alveolar. The cisternal milk fraction located in large mammary ducts and cisternal cavities is immediately available for milking. The alveolar milk fraction located in small milk ducts and alveoli is fixed by capillary forces and requires the milk ejection in order to be forcefully expulsed into the cisternal cavities to be available for milking. In cows, the cisternal milk fraction after an interval of 12 h from previous milking is 20% (Figure 2). Cisternal fraction in cows after 12 h milking interval was 5.1 kg in early and 2.6 kg in late lactation (Knight et al., 1994). Milk removal of the cisternal fraction is performed by surmounting the teat sphincter barrier.

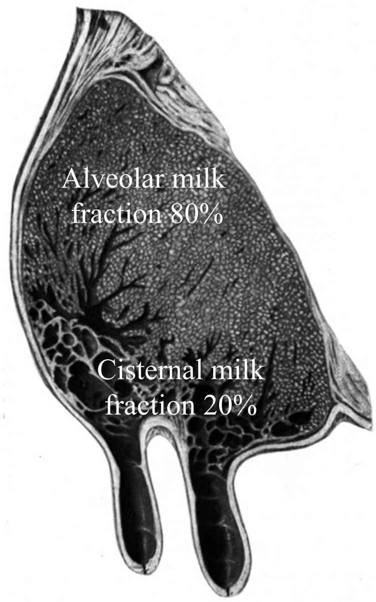

Figure 2. Cisternal and alveolar milk partitioning in the cow before milking (Nickel et al., 1976)

The cisternal milk fraction increases slowly between 4 to 12 h after milking. Thereafter it increases more rapidly to a plateau after 20 h while the increase of the alveolar fraction is more rapid reaching a plateau after 16 h (Knight et al., 1994; Davis et al., 1998; Ayadi et al., 2003). The cisternal milk fraction is increasing with the number of lactations (Bruckmaier et al., 1994c). The cows with larger cisternal size are associated with a smaller milk yield decrease than cows with smaller cisternal size during once daily milking (Knight and Dewhurst, 1994; Stelwagen and Knight, 1997). The reason could be an autocrine inhibitor of milk secretion (Wilde and Peaker, 1990) which is active in milk stored in the secretory tissue and not in milk stored within the gland cistern (Henderson and Peaker, 1987). Therefore, large-cisterned cows store their milk mainly in the cisternal area where the inhibitor is inactive. In contrast, cows with smaller cisternal size increase the most their milk production when milked thrice daily (Dewhurst and Knight, 1994).

Cisternal milk fraction in most of the dairy ewe breeds is usually larger than 50% (Figure 3; Caja et al., 1999; Rovai, 2000; Marnet and McKusick, 2001).

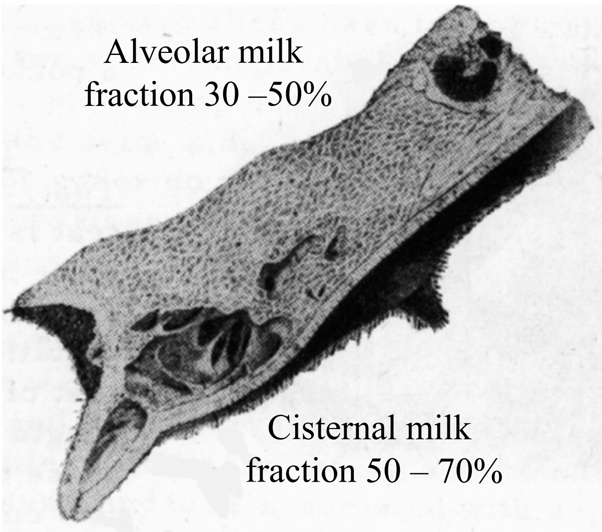

Figure 3. Cisternal and alveolar milk partitioning in the ewe before milking (Turner, 1952)

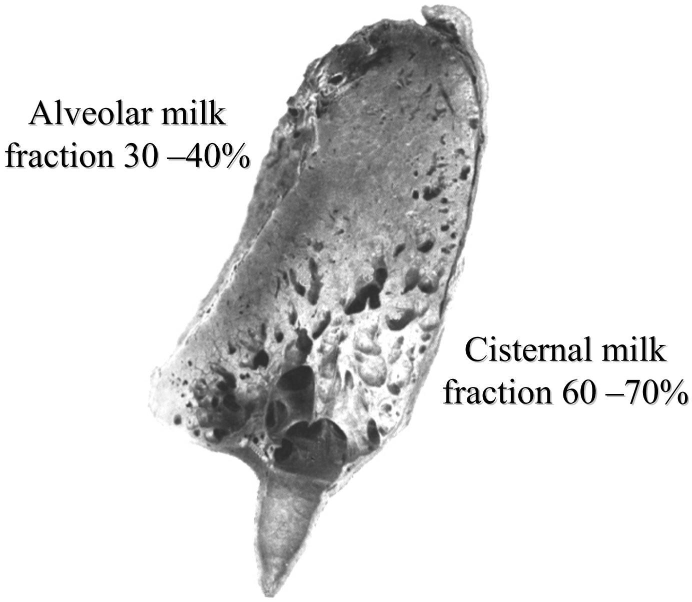

Figure 4. Cisternal and alveolar milk partitioning in the goat before milking (Nickel et al., 1976)

Similarly a large cisternal milk fraction was found in goats (Figure 4; Bruckmaier and Blum, 1992; Marnet and McKusick, 2001). Therefore in ewes most of the milk is located within the cisternal cavities in the ewes. The milk removal process differs from cows, because of different anatomical conditions and different partitioning of the alveolar and cisternal milk fractions. The cisternal milk fraction starts to fill up immediately after the previous milking, and linearly increases for at least 16 h (Peaker and Blatchford, 1988). In goats as well as in cows, the cisternal fraction is filling up, before the alveolar milk fraction reaches its maximum (Knight et al., 1994). The only difference between the goats and sheep is that the teats are not always placed vertically in ewes. Therefore, milk portion below the teat orifice can be collected only during stripping (Bruckmaier et. al., 1997; Labussière, 1988).

1.3. B-mode ultrasound imaging history of mammary cavities

B-mode (B = brightness) ultrasonography generates cross sections of body tissues according to the amplitude of the reflected signal (Figure 5). Different reflection intensity represents different levels of brightness on the ultrasound screen. Therefore, black fields on the screen are created from substances such as clear fluids which do not reflect, while non-homogenic tissues appear in grey colour. This technique was used to detect pregnancy in sows, ewes, goats and cows. The mammary gland ultrasound imaging was first performed for the teat area (Cartee et al., 1986; Worstorff et al., 1986). B-mode ultrasound images in a water bath taken vertically with the axis longitudinally through the teat canal of the total mammary cisternal area in cows, goats and sheeps were performed by Bruckmaier and Blum (1992). Another technique for the mammary gland ultrasound measurements was used by Ruberte et al. (1994) via acoustic coupling gel which is placed on the portion of the intermammary groove caudally to the mammary glands. All these studies show that the B-mode ultrasound imaging technique is suitable for evaluation of the cisternal cavities in different mammary species.

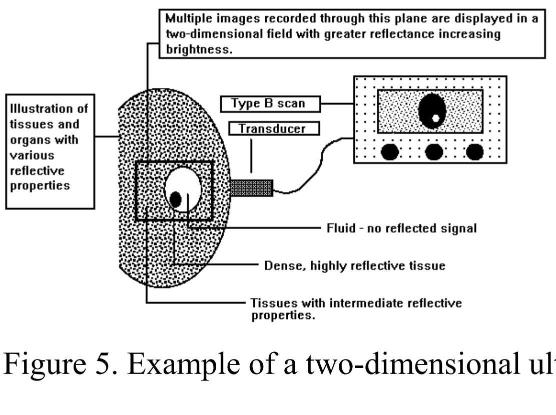

Figure 5. Example of a two-dimensional ultrasonic B-scan (Edgarton, L.A., 1992)

1.4. Oxytocin and milk ejection

Oxytocin (OT) is a nine amino acid peptide that is synthesized in hypothalamic neurons and transported down axons of the posterior pituitary for secretion into blood. It mediates three major effects in a female: stimulation of milk ejection, stimulation of uterine smooth muscle contraction at birth and establishment of maternal behaviour. OT is synthesized in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus and secreted from the axon terminals of the SON and PVN nucleus in the posterior pituitary gland (Crowley and Armstrong, 1992). In the SON OT neurosecretory cells are preferentially found in the rostral and dorsal parts of the nucleus and PVN OT neurons in the medial magnocellular division (Armstrong et al., 1980; Rhodes et al., 1981). OT synthesis from the neurohyphophysis is Ca^{2+} dependent occurrence. It is normally induced by the depolarization of secretory terminals by invading action potentials from magnocellular neurosecretory neurons (Crowley and Armstrong, 1992).

The posterior pituitary gland releases OT after increased activity of the OT neurons during lactation. Discharge of the neurosecretory granules occurs via exocytosis, i.e., during the terminal depolarization within the posterior pituitary lobe, and OT is released into the blood circulation (Crowley and Armstrong, 1992).

A larger number of OT receptors on myoepithelial cells could potentiate the effect of OT release. However, the number of OT receptors does not change during lactation (Soloff et al., 1982). The time needed that OT released from the posterior pituitary reaches the mammary gland through blood circulation is on average 24.3 s in the goat, 16.9 s in the ewe and dosage dependent in the cow from 16 to 29 s for 1 or 0.1 IU, respectively (Martinet et al., 1999). OT is eliminated from blood through kidneys and liver in the rat, rabbit, cow and ewe (Schmidt, 1971).

The milk ejection reflex is a neuroendocrine reflex that is not under conscious control of the animal. It was first described by Ely and Petersen (1941). It occurs in response to the tactile stimulation of the mammary gland through neuroendocrine reflex arc (Crowley and Armstrong, 1992; Lincoln and Paisley, 1982). During the pre-milking preparation process the stimulation of the teat activates neural receptors that are sensitive to pressure and impulses are carried via the inguinal canal to the lumbar nerves. These lumbar nerves connected by posterior (dorsal) roots terminate at the spinal cord. From there the signal is transmitted to the SON and PVN of the hypothalamus. When OT is released from the posterior pituitary it travels through the blood circulation up to the myoepithelial cell OT receptors. The OT receptors on myoepithelial cells respond to elevated OT concentrations with induction of myoepithelial cell contraction. Consequently, alveolar milk is shifted into the large ducts and there from into the cistern (Bruckmaier, 2001). This causes rapid increase of the intramammary pressure within the cistern (Bruckmaier and Blum, 1996) and an enlargement of the cisternal area (Bruckmaier and Blum, 1992). However, myoepithelial contraction is not controlled by the nervous system. If the OT concentration rises above the threshold level (3-5 ng/l), milk ejection up to a maximum intramammary pressure occurs (Schams et al., 1984; Bruckmaier et al., 1994b). Additional contraction occurs only if supraphysiological amounts of exogenous OT are injected. This is mainly used experimentally to determine the milk remaining in the udder (residual milk) after milking. It is known that the lag time from the start of tactile teat stimulation until the start of milk ejection ranges from 1 to 2 min (Bruckmaier and Hilger, 2001). The OT concentration throughout the milking process must remain elevated above the threshold level to continue milk ejection until the end of milking. Milk ejection throughout the milking is important for complete milk removal in cows (Bruckmaier et al., 1994b). Therefore, a teat stimulation with liner causing OT release is not only required at the beginning of the milking, but throughout the entire milking. Teat stimulation causes OT release which remains similar or increases during the course of lactation, while milk ejection is delayed in late lactation when milk yields decrease (Mayer et al., 1991) or at low udder filling (Bruckmaier and Hilger, 2001). The milk ejection starts and course after the tactile teat stimulation showed no differences in high and low yielding cows in a similar lactational stage (Wellnitz et al., 1999) and udder filling (Bruckmaier and Hilger, 2001).

The milk ejection is partially or totally blocked when the cow is under stress causing elicited blood epinephrine levels. This interferes with nerve impulses in SON and PVN and subsequent OT release. Cows milked in unfamiliar surroundings reduce or totally abolish OT release (Bruckmaier et al., 1993; Bruckmaier et al., 1996; Rushen et al., 2001).

Cisternal milk fraction and cavities are larger in goats and sheep compared to cows (Bruckmaier and Blum, 1992). Large cisternal fraction causes long period of cisternal milk flow without interruption of the milk flow. It is known that alveolar milk ejection in goat and sheep occurs in response to elevated OT concentrations (Bruckmaier and Blum, 1992; Heap et al., 1986). Anyhow, breed specific delay in OT release during milking is obvious (Bruckmaier et al., 1997).

1.5. Machine milking

1.5.1. Conventional milking in cows, mares, goats and ewes

Optimally machine milking removes quickly and completely secreted milk with good hygiene maintaining high milk yield and animal health at a low cost (Bruckmaier and Blum, 1998). Proper milking routine should provide unstressful environment for the cow and the farmer, and ensure that the pre-milking teat preparation is done in the same sequences of events to result in complete milk ejection before the milking starts and to minimize the amount of milk that should be removed by stripping. The milking routines directly affect milk ejection and therefore the amount of alveolar milk that can be collected during the milking process. Without milk ejection only cisternal milk fraction can be collected during milking. Manual teat stimulation or action of the liner during milking evokes OT release which causes alveolar milk ejection. OT release above the threshold of 3 – 5 ng/l is sufficient to evoke maximum milk ejection. Once the OT concentrations are above threshold, no additional effect of high OT concentrations is documented (Schams et al., 1984). Therefore, the right timing of the OT release is more important than the absolute concentration. Milking on empty teats can occur at the start of milking in case of too short pre-stimulation. Moreover, milking on empty teats reduces milkability during further milking, even after occurrence of delayed alveolar milk ejection (Bruckmaier and Blum, 1996). Optimally the milking machine should be attached shortly after pre-stimulation if milk ejection is evoked. However, not the entire amount of milk can be removed from the udder. During normal machine milking about 90 % of the stored milk can be removed through the action of endogenous OT (Knight, 1994; Bruckmaier, 2003).

The remaining residual milk can be collected only with administration of supraphysiological dosage of OT (Knight, 1994; Bruckmaier, 2003).

1.5.2. Automatic milking in cows

Milking routines in automatic milking systems (AMS) differ from those in conventional milking. Cows are voluntarily milked throughout the day at variable milking intervals. Teat cup attachment requires usually more time than in conventional milking (Hopster et al., 2002; Macuhova et al., 2003). The teats are cleaned in AMS by brushes or rollers sequentially, by a horizontal rotating brush simultaneously, in the same teat cups as used for milking simultaneously, by a separate cleaning device sequentially (De Koning et al., 2002). The teat cup attachment procedure is longer than in the conventional milking system, when the milker attaches the teat cups. Additionally, the success rate can vary between individual milkings. The minimum attachment time of all 4 teat cups varies in the AMS depending on the milking robot used and can be 36 s as reported by Ipema (1996) or 66 s as found by Hopster et al. (2002). In a multi-box AMS the start of teat cup attachment can be delayed after the end of teat cleaning, while cow has to walk between cleaning and milking box (Macuhova et al., 2003).

1.6. Milking characteristics

Milk flow rate is a function of teat anatomy and mechanical properties of the milking machine. The patterns of the milk flow rate are rather repeatable in an individual cow from day to day and even in succeeding lactations. The milk flow consists of three phases (see Figure 6): a short and immediate increase (phase 1), a plateau period which is rather constant (phase 2) and a period of declining slope (phase 3), when individual quarters are with little or no milk.

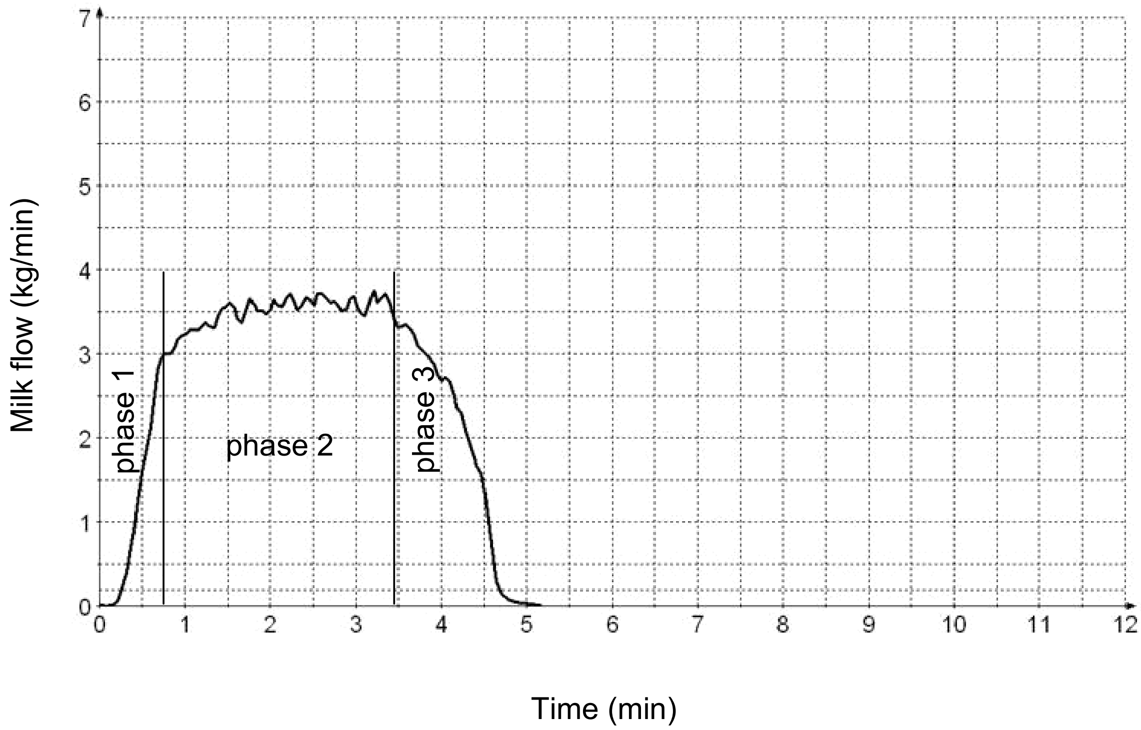

Figure 6. Milk flow curve with short immediate increase (phase 1), plateau (phase 2) and period of decline slope (phase 3) of the whole udder during conventional milking

In conventional milking where all teat cups are removed at once, the milk flow pattern is highly influenced by the milk flow pattern of individual quarters. The milking time depends not only on the milk flow rate, but also on the milk yield that has to be removed during the milking process. In AMS teat cups are removed individually and therefore there is a possibility of milking on empty teats only during the first minute of milking. Premilking teat preparation for 1 min compared to treatment without premilking teat preparation decreases machine-on time and increases peak flow rate (Bruckmaier and Blum, 1996). Total milk yield, average flow rate, time to reach plateau and time to reach peak flow rate did not differ between the treatments (Bruckmaier and Blum, 1996). Maybe, the elastic tissue or muscles surrounding milk lobes and cisternal inlets as well as the streak canal muscles are more relaxed after a longer pre-milking teat preparation. This relaxation could cause an easier milk flow from alveoli through the ducts and out of teats. In early lactation teat cups could be attached even if milk ejection is not evoked, while a large portion of cisternal milk exists.

The attachment delay is defined as time interval between the start of preparation and attachment. Machine-on time decreased with a longer attachment delay in American Holstein cows (Rasmussen et al., 1992). The attachment delay influenced milk yield, milk composition, or residual milk and fat in both American Holstein and Danish Jersey cows. Milking without pre-stimulation tended to be longer (5.9 min) compared to normal milking with the udder washing procedure (5.2 min) (Momogan and Schmidt, 1970). They found that OT release and presence in the bloodstream are transitory. Elimination of udder washing caused a delayed OT release and longer milking time. Although liner alone was able to induce OT release and milk ejection within 1 to 2 minutes after teat cup attachment.

1.7. Objectives of the present study

Milking routine causes OT release, with subsequent milk ejection and milk removal in mammal species. There is scarce information about optimal milking routines in mares, cows and ewes. Therefore, the aim of this study was to investigate the influence of several milking routines on the milk ejection and milk removal in mares, cows and ewes. In Chapter 3.1 optimal milking routines which cause OT release, and milk ejection in the mare were tested (Dzidic et al., 2002). Chapters 3.2 and 3.3 deal with question of evaluating optimal milking routine for two different dairy cow breeds in two different AMS and optimal timing of teat cup attachment (Dzidic et al., 2004a; Dzidic et al., 2004b). Chapter 3.4 evaluates the question of the optimal morphological characteristics to ensure complete milk removal in the Istrian dairy crossbreed ewes (Dzidic et al., 2004c).

2. MATERIALS AND METHODS

2.1. B-mode ultrasound imaging

The udders of mares were visualized by B-mode ultrasound imaging using a method developed for cows, sheep and goat mammary glands (Bruckmaier and Blum, 1992). The mare udder was immersed into a plastic bucket, and the picture was taken from below the teat in ventral direction (Dzidic et al., 2002). The picture was taken before and after milk ejection induced by OT (10 IU i.v.). The cisternal areas were measured using a digitizing tablet with a special computer program (Sigma-Scan, 1988).

2.2. Oxytocin radioimmunoassay

The OT concentration determination in blood plasma was performed according to the radioimmunoassay (RIA) which was developed and modified at the Institute of Physiology, Weihenstephan, TU-München (Schams et al., 1979; Schams, 1983). The OT was extracted from blood plasma using cartridges (SEP-PAK C_{18}), where polar compounds are extracted before non-polar compounds. At the start of extraction cartridges were saturated with 2ml of methanol and 5 ml distilled water. Thereafter, 1 ml of plasma diluted with 2 ml of 0.05M phosphate buffer (ph 7.5) was applied to the cartridge. The gamma globulins and most of the proteins were eluted. The cartridge was flushed with 4 ml of acetic acid 1.5% (pH 4.85). The OT was extracted with 2 ml of tetrahydrofurane (Merck, Germany) and dispensed in glass tubes. The solution was evaporated. The residue was dissolved in 0.5 ml of buffer and used for the RIA. The RIA principle is based on competition between a radioactively labelled and free OT for specific antibody which is possible only through limited number of binding sites. The OT is radioactively labelled, when one H-atome in Tyrosine from an OT molecule is replaced by ^{125}Jod . The assay is performed in tubes using 0.05M phosphate buffer with 50 mM EDTA, and 0.5 g/l human albumin (pH 7.5) used as diluent. The OT extract (200 µl) was incubated for 24 h at 4-6˚C with antiserum (100 µl) in a final dilution. During the assay duplicate (unknown) or triplicate (standard) determinations were done. The labelled OT (3000 CMP) mixed with 100 µl buffer was added to tubes and incubated for 48 h. Thereafter, the horse serum (0.1 ml) was diluted with phosphate buffer at a ratio of 1:4 and 1 ml of a 2g/l suspension of charcoal in phosphate buffer. All tubes were thoroughly mixed and centrifuged for 20 min at 4˚C. The supernatant was removed and counted for 4 min. The binding activity of serum containing antibodies was determined in a gamma-counter. The OT concentration was determined after the standard curve for the plasma samples with predetermined OT concentration was done. The lower limit for the OT concentration using this assay was 0.25 – 1.0 pg/ml.

2.3. Milk flow measurement

2.3.1. Strain gauge system

A strain gauge system was first used to measure the milk flow of cows (Schams, et al., 1984; Bruckmaier et al., 1992). Milk was collected in a bucket or jar that is suspended to a strain gauge. The strain gauge continuously measures weight using a Wheatstone's bridge, with the time interval of 1.2 s (Dzidic et al., 2002). This weight increase in time was then transformed into the actual milk flow rate and shown as a milk flow curve on a strip chart recorder. The milk flow curve from the strip chart recorder can be visualised using a digitizing tablet with a special computer program (Sigma-Scan®, 1988). The strain gauge system is not very mobile, but it is independent of the milk composition and provides extremely exact measurements. This system is mainly used when milking is performed in a bucket.

2.3.2. Lactocorder

The Lactocorder® (WMB AG, Balgach, CH) consists of a hydraulic module which performs measurements and an electronic module which processes and saves the data (Figure 7). The pulsating milk is transferred through the centrifugal head to the 60 individual electrodes which measure electric conductivity of the milk every 0.7 s. During each measurement the date and time when the measurement was done are written in the corresponding file. Recently, a single quarter milk flow curve measuring system was developed with four Lactocorder systems (Wellnitz et al., 1999). This system used in AMS directly records into a computer program the milk flow data from each quarter every 2.8 s (Macuhova et al., 2003). The Lactocorder system is mobile and easy to use.

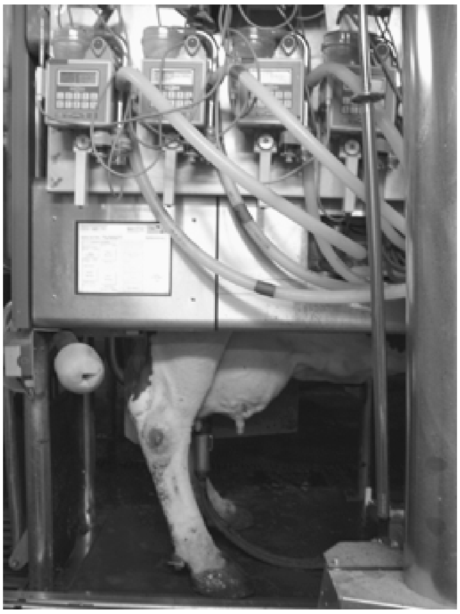

Figure 7. Quarter lactocorders built in an automatic milking system between the teat cups and shut off and regulator valves

2.3.3 Udder morphology measurements in ewes

The udder volume size in the ewe was estimated using the water displacement method initially developed for goats (Bruckmaier et al., 1994a). The ewe udder was dipped into the water-filled bucket before the evening milking once in mid-lactation. The water displacement was measured for each of the ewes. The udder shape was evaluated using the linear scale system from 1 to 9 (De la Fuente et al., 1996). The udder shape scored from 1 (faulty for machine milking) to 9 (ideal for machine milking; Figure 8).

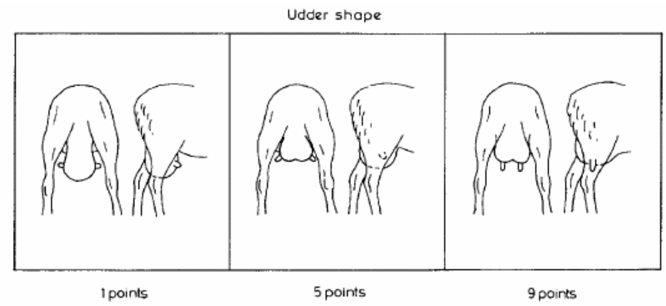

Figure 8. Linear scale system for udder shape evaluation where udder shape 1 represents udder faulty for milking and udder shape 9 ideal for milking (De la Fuente et al., 1996)

The teat length was measured from the teat base until the teat orifice and the teat angle was measured as an angle which the teat closes with the vertical line of the udder.

2.3.4 Experimental protocols

Experiments in the first study were performed during machine milking in mare (see Dzidic et al., 2002). The mare udder cisterns were visualized by B-mode ultrasound imaging, milk flow was recorded using a strain gauge system with differentiation unit and strip chart recorder and blood samples were collected for the OT determination. In experiment I mares were milked after the 1 min routine prestimulation. Milking 2 started after the break of 15 min. At the end of milking 2, 10 IU (i.v.) of OT were injected to remove residual milk. In experiment II removal of residual milk was performed already after first milking. Milking 2 was performed after a 15 min break from the end of residual milk removal.

In the second study, five treatments were applied in cross-over design to 45 cows during AMS milking (see Dzidic et al., 2004a). Each treatment represented one pre-milking teat preparation routine as described in Dzidic et al. (2004a). On five additional days blood samples were taken during milking from 10 cows. During all experimental milkings quarter milk flow curves were recorded using an especially rebuilt set of four Lactocorders (WMB, Balgach, Switzerland).

Third study was performed during AMS milking, where 62 cows were assigned to four different treatments during three periods in experiment I as shown in Dzidic et al. (2004b). Each treatment included different pre-milking teat preparation duration and cleaning water temperature. In experiment 1 and 2 milking characteristics were measured using the especially constructed set of quarter Lactocorders. In experiment 2 ten cows were randomly assigned to the treatments and blood samples for OT determination were taken during experimental milkings.

Last study in this thesis was performed during machine milking of 63 crossbreed ewes (Dzidic et al., 2004c). Milking characteristics were evaluated in early, mid and late lactation with specially calibrated Lactocorder, while udder morphology was evaluated once in mid-lactation.

3. RESULTS AND DISCUSSION

3.1. Mare and machine milking

To the best of our knowledge there was no information on the cisternal size in the mare mammary gland. Therefore, the first step was to determine the size of the cistern in the mare. Mare average total cistern size obtained by ultrasound was 18 ± 1 cm^2 (Figure 9). After the OT application, a cistern area enlargement of 38 ± 12 % was observed (Figure 10). Thus, like in other domestic mammals, milk ejection caused an enlargement of the cistern cavities. The cisternal size is quite similar to that of sheep and goats, while smaller than in cows (Bruckmaier and Blum, 1992).

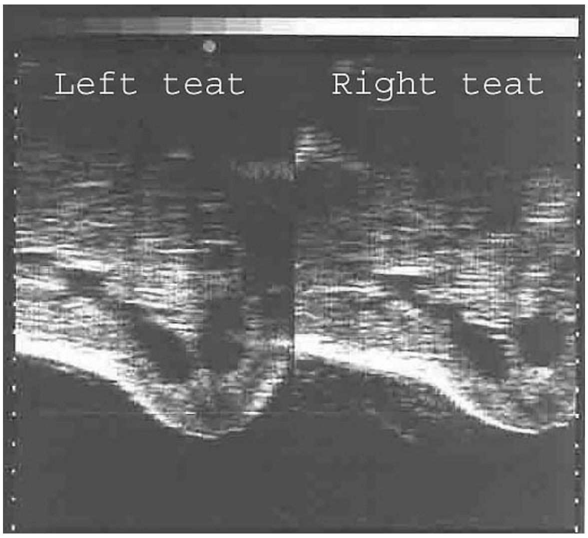

Figure 9. B-mode ultrasound cross section of left and right Süddeutsches Kaltblut breed mare teat

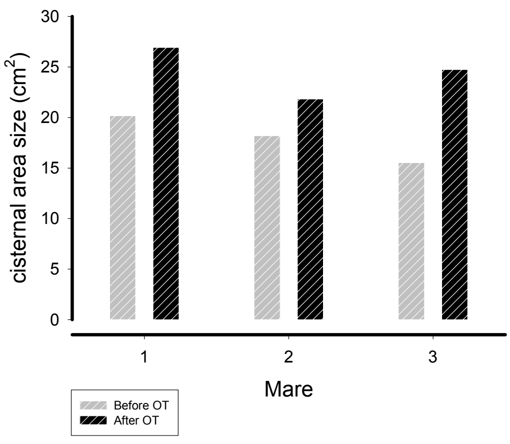

Figure 10. Cisternal area size before and after 10 IU (i.v.) OT administration in three Süddeutsche Kaltblut breed mares

OT release was present mainly during pre-stimulation and followed by a milk flow rise during the first minute of milking (Figure 11). The OT release during machine milking of mares is obviously insufficient for complete milk removal from the udder, while after removal of the cisternal fraction only small portion of alveolar milk was removed. The basal OT concentration is quite similar to that of cows around 4 pg/ml. During pre-stimulation the OT concentration rapidly rises and during machine milking it decreases. The amount of residual milk is 43% after the normal milking routine. In cows it is known that the amount of residual milk can be 10 to 30% (Bruckmaier and Blum, 1998). It can be speculated, because mares were also suckled by their foals, that this is the reason why such a high amount of residual milk was obtained after milking. Suckling in cows resulted in greater short-term increase in milk production than milking, although calf removal resulted physiological disturbance during further milking (Bar-Peled et al., 1995). Suckling by an alien calf or separation of the own calf causes a failure of OT release (Tancin and Bruckmaier, 2001). Similar problems with OT release occur when cow which was several weeks only machine milked is first time suckled (Tancin and Bruckmaier, 2001). The other possibility could be that Süddeutsches Kaltblut breed is not well adapted to the machine milking.

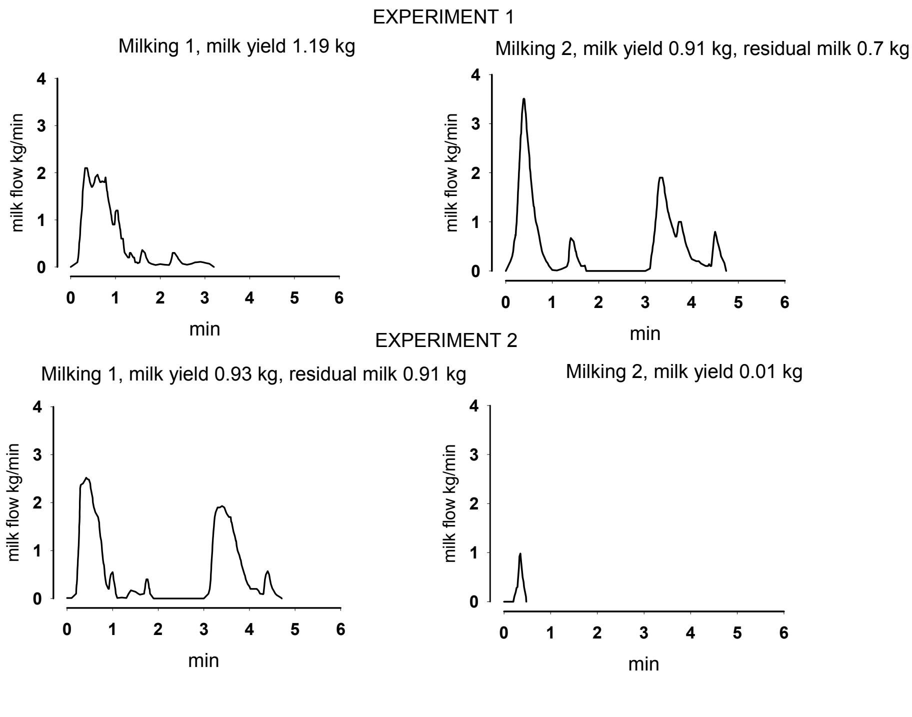

Figure 11. Milk flow curves in experiment 1 and 2 of Süddeutsches Kaltblut breed mare nr. 3 during normal milking and milking after 10 IU (i.v.) OT administration

3.2. AMS and sequential teat cleaning by brushes

Because of variable milking interval throughout various lactation stages, a unique measurement was estimated, representing both variables in one. The degree of udder filling was estimated as a percentage of actual milk yield compared to the maximum storage capacity. The maximum storage capacity of the mammary gland was estimated as the highest milk yield obtained at one successful milking (with milking interval not longer than 12 h) in month 2 of the respective lactation. Cows visited the AMS mostly when their udder contained 40 – 80% of milk (Bruckmaier and Hilger, 2001). Although about 5% of milking occurs at a very low degree of udder filling, when the cows need longer pre-stimulation in order to achieve alveolar milk ejection at the start of the milking.

De Koning and Ouwetjes (2000) found 14.9% of milkings with the milking interval shorter than 6 h. The importance of the adequate pre-milking teat preparation at a low degree of udder filling is therefore obvious. During milking similar amount of OT was released independently of the pre-milking teat preparation applied. It is obvious that only the timing of the OT release is important, not the amount of OT. A similar situation occurred with the time until start of the milk flow, which was the shorter with a more filled udder than with a less filled udder. Bruckmaier and Hilger (2001) showed that the alveoli need more time to contract when they are partially filled. The peak flow rate was not influenced by the amount of time spent on pre-milking teat preparation, while the average flow was reduced in less filled udders which were not adequately pre-stimulated. A similar phenomenon was already observed in conventional milking system (Rasmussen et al., 1992). A bimodality, showing delayed milk ejection when cisternal milk fraction is present was detected when any of the quarter milk flow curves had a flow pattern with two increments separated by a clear drop of milk flow below 200 g/min shortly after the start of milking. The bimodality was present when the time spent on pre-milking teat preparation was lower than 1 min (Figure 12).

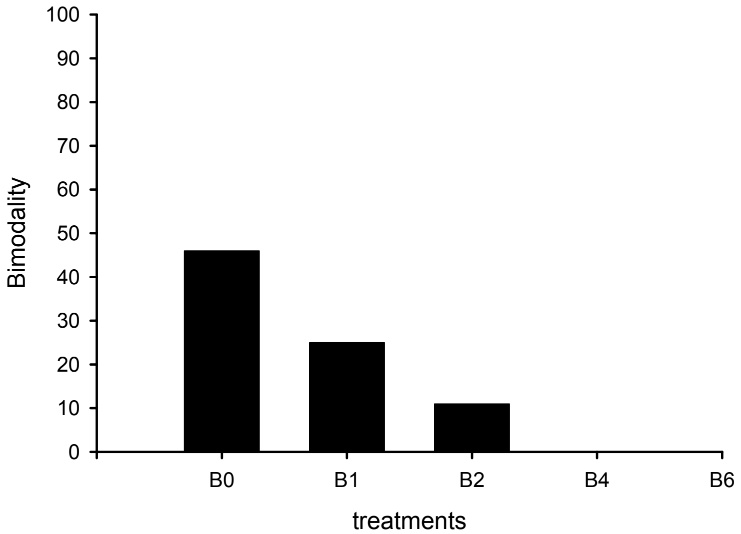

Figure 12. Frequency of bimodal curves in treatments without brushing (B0), with one brushing cycle (B1), two brushing cycles (B2), four brushing cycles (B4) and six brushing cycles (B6) during milking in a single stall automatic milking system (n=135)

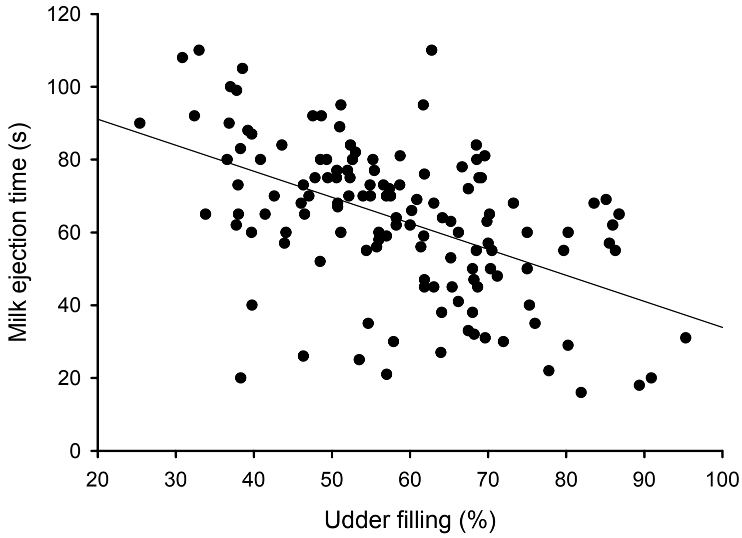

Figure 13. Relationship between milk ejection time in the treatment without pre-milking teat preparation and different percentage of the udder filling (n=62)

In a previous study (Bruckmaier and Hilger, 2001) earlier occurrence of the alveolar milk ejection in the conventional milking system was observed in a more filled udder with milk compared to udders containing less milk. A similar trend was observed in our study of the AMS herd (Figure 13). Therefore, when the udder is less filled with milk, i.e. less cisternal milk is present in the udder, more time is needed to be spent on pre-milking teat preparation so that empty teats milking does not occur.

3.3. Effect of cleaning duration and water temperature in AMS

The period of teat cleaning is ideal for pre-stimulation and udder hygiene. Brushing of teats and udder for 60 s and teat cup attachment in multi-box AMS induce OT release and milk ejection (Macuhova et al., 2003). The teat cleaning with a towel for 75 s resulted in no bimodal milk flow curves, i. e. delayed milk ejection, of any quarter (Bruckmaier et al., 2001). However, if teat cups were attached without teat cleaning, first attached quarter resulted in bimodal milk flow curve. More cisternal milk is present in early lactation, therefore the teat cups can be attached to the teat even before the milk ejection occurred, while machine milking on empty teats is less likely to occur (Rasmussen et al., 1992).

The time from the start of pre-milking teat preparation until the increase of intramammary pressure is 1.3 min and 1.7 min in early and late lactation, respectively (Mayer et al., 1991). In early lactation more cisternal milk is available than in late lactation (Knight et al., 1994; Pfeilsticker et al., 1996). During milking at low udder filling after a short milking interval or in late lactation, the cisternal fraction is small or missing and additionally alveolar milk ejection is prolonged (Bruckmaier and Hilger, 2001). Furthermore, attachment does not occur immediately after teat cleaning in case of multi-box AMS, when the milking box and the teat cleaning box are separated (Macuhova et al., 2003). If teat cleaning and milking are integrated in the multi-box system, there is no additional delay between teat cleaning and attachment (Sonck and Donkers, 1995). Conditioned stimuli during studies in the multi-box AMS was not observed, although a positive effect of providing concentrate during pre-stimulation and milking was shown to have a positive effect on OT release and subsequent milk ejection (Johansson et al., 1999).

In our experiment we had fixed the minimum (5 h) and the maximum interval (depending on the milk production per cow). However, we observed a shift in the milking interval throughout lactation. Bimodal curves showing delayed milk ejection were observed only in treatment without pre-milking teat preparation. The time from the start of pre-milking teat preparation until the first teat cup was attached (preparation lag time) was longer than 80 s. The optimal preparation lag time in conventional milking was 60 to 90 s (Rasmussen et al., 1992). Milking time was longest in treatment without pre-milking teat preparation, although the total milking time, i.e. milking time including pre-milking teat preparation, was not reduced. The only milking characteristic that was considerably lower during milking without pre-milking teat preparation was the average flow. The average flow rate was lowered especially during a milking interval shorter than 8 h. Before milking started OT concentrations remained low, showing no conditioned stimuli prior to milking. Already after 30 s of milking OT concentrations were elevated in treatment without pre-milking teat preparation. In all other treatments peak concentrations were already observed already during the pre-milking teat preparation procedure. During milking all OT concentrations remained elevated above the threshold level and there were no differences between the treatments in OT release during milking. Cold water usage in AMS milking routine did not influence OT release, milk ejection or milking characteristics. However, the udder health should be taken into consideration when using cold instead of warm water in the AMS milking routine. Wet pre-milking treatment is usually associated with increased hyperkeratosis and incidence of intramammary infection (Barkema et al., 1999). Milking routine with cold water in our study included a drying-off phase. Drying of the teats was crucial after the wet phase with water or premilking disinfection dip, causing a reduction in bacterial counts on teats (Galton et al., 1986).

3.4. Udder morphology and milking characteristics in ewes

Three different Istrian dairy crossbreed ewes (with 75% Istrian and 25% Awassi, IAI; with 25% Istrian, 25% Awassi and 50% East Friesian, IAEF; and with 50% Istrian and 50% Awassi, IA) were evaluated for their milkability and udder morphology during one lactation. These crosses give 296 kg of milk in 203 days of lactation. The best average production per day was found in IAEF crosses.

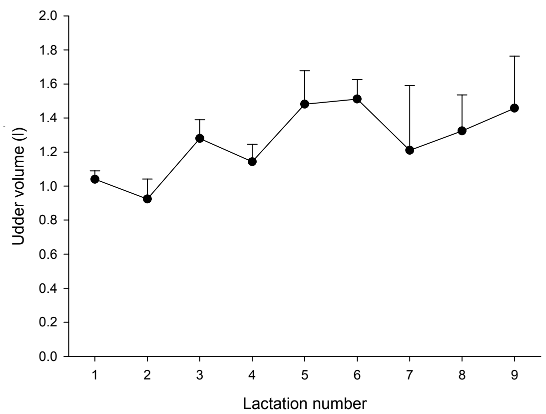

Figure 14. Udder volume change as a function of lactation numbers in Istrian dairy crossbreed ewes (n=63)

Three types of milk flow curves were observed in this study (see appendix 9.4). First type of milk flow could occur with and without alveolar milk ejection (Mayer et al., 1989; Bruckmaier et al., 1997). Crosses with more Istrian blood might have more cisternal than alveolar milk within the first peak in the milk flow curve. Although, OT or intramammary pressure (IMP) measurements could answer this question. Milk flow curves with two peaks are the second type of milk flow profiles, where first peak represents cisternal milk fraction and second alveolar milk fraction. Third type of the milk flow curve represent weak or absent OT release during milking with a peak flow rate below 0.4 kg/min (Bruckmaier et al., 1997). Proposed milk flow classification seems to be feasible for the evaluation of East Friesian ewes (Bruckmaier et al., 1997) and their crosses. The increase in the udder volume with increasing parity was observed in this study (Figure 14). On the contrary, the teat angle and teat size did not change. The IAEF crosses had the highest peak flow rate and milk yield, with shortest teats, highest teat angle and biggest udder volume. There was no ideal milking udder found in this herd, although the average udder shape score was 4.3. It seems likely that, with the selection process only the volume of the udder and milking characteristics were changed and no improvement in udder morphology towards the machine milking was observed. Moreover, Machega and French Rouge de l'Ouest ewes had a better teat position for milking as compared to more milk producing Lacaune breed (Such et al., 1999; Malher and Vrayala-Anesti, 1994). The present study showed that the udder measures used together with milk emission kinetics could be a good pool for further selection of Istrian dairy crosses.

4. CONCLUSIONS AND RECOMMENDATIONS

Although OT release with subsequent alveolar milk ejection occurs shortly after pre-milking teat preparation in mare, ewe and cow, anatomical conditions should be taken into consideration to ensure complete milk removal.

Anatomical conditions in cattle are different from those in mare and ewe, although milk ejection in response to elevated OT concentration is similar. Although goats and sheep have large cisternal milk fraction, teat orifice in sheep is located above the edge of the gland. Therefore, udder morphology measures in sheep are important in respect to complete milk removal, while additional handling is needed to collect cisternal milk fraction which is below the teat orifice. Udder volume and teat angle showed more pronounced influence on milking characteristics than teat size. Udder morphology is less crucial in cows and mare, while the teat orifice is located at the bottom of the gland and no additional handling is needed to collect milk from the cisternal gland.

In mare and sheep milking, mainly the cisternal milk can be collected throughout the milking process. Most probably the action of the liner throughout the milking process is not sufficient to evoke or maintain already established milk ejection. Additionally, mares are usually milked and suckled by the foals. Therefore, it can be recommended to study the suckling and milking relationship in respect to the alveolar milk ejection and possible inhibition of the milk ejection reflex. Further selection could possibly ease the removal of the alveolar milk during the milking. On the contrary, in cows the induction of alveolar milk ejection before the start of milking avoids interruption of the milk flow after removal of the cisternal milk. However, with the introduction of automatic milking systems, udders which are less filled with milk should be milked after prolonged pre-stimulation and therefore, it can be recommended to optimize the pre-milking duration of teat preparation according to the actual level of milk present in the udder which could increase the capacity of the AMS.

Baseline OT concentrations were low in mares and cows before the start of milking, showing no conditioned OT release. Mares released OT mainly during the pre-milking teat preparation process and therefore only a part of the alveolar milk was collected during milking. On contrary, cows released OT throughout the milking process which is obviously necessary for complete udder evacuation.

If pre-milking teat preparation in cows is performed with water, as in an AMS, milk ejection or milk removal is not different if the cleaning is performed with warm or cold water.

This finding allows saving energy for water heating. However, further investigations are needed.

To conclude, pre-milking teat preparation resulted in increased OT release with subsequent milk ejection in all species investigated. However, duration of OT release was much shorter in mares compared to cows, while pre-milking teat preparation provided better stimuli than the action of the liner during the milking. Immediate OT release prior to milking does not cause interruption of the milk flow in ewe as in cow milking, while cisternal fraction is larger and lasts until alveolar milk fraction is forcefully shifted into cisternal cavities. Ewe breeding towards more milking production while respecting optimal udder morphology resulted in higher milk production with fast milk removal. Moreover, results in ewes and especially mares are basic observations in one breed or crossbred and therefore cannot be generalized. On contrary, results in cows during milking in a single stall AMS are rather complete and we can say that the pre-milking cleaning routine is able to elicit OT concentration with subsequent milk ejection prior to milking. The usual delay between teat cleaning and sequential teat cup attachment did not negatively influenced milk ejection and milk removal. Individual adaptation of pre-milking cleaning routine to the actual degree of the udder filling can reduce total milking time. Knowledge collected in this work is sufficient to optimize milking routines during cow milking in a single stall AMS milking, while in mare and ewe milking milking routines could be optimized only for observed breeds.

5. ABSTRACT

Before milking, only milk stored in cistern is immediately available for removal. Alveolar milk is available only after milk ejection occurs in response to tactile teat stimulation and OT release. Tactile teat stimulation is performed during the pre-milking teat preparation process. Continuously elevated OT concentrations are necessary for complete milk removal.

This work comprises information about the requirement for pre-milking stimulus in several dairy species to ensure OT release, milk ejection and complete milk removal.

Measurements included plasma concentrations of OT during mare and cow milkings. Milk flow patterns were recorded during mare, ewe and cow milking.

Pre-milking teat preparation caused milk ejection before the milking started in mares, cows and ewes. Anyhow, there are some differences between the species. Mares released high OT concentrations only in the pre-milking teat preparation phase. On contrary, cows milked in an automatic milking system (AMS) released OT already 30 s after the start of pre-milking teat preparation. Moreover, the teat cup liner was suitable to stimulate further milk ejection and therefore OT concentrations remained elevated throughout the milking process. However, the liner was not able to replace pre-milking teat preparation in mares. Milk ejection in combined suckling – milking regime is still not clearly defined. Ewes have larger cisternal cavities, if milk ejection does not start immediately at the start of milking, interruption of the milk flow would not occur.

Mares, cows and ewes are usually milked in a stable with a milking machine or in a milking parlour. However, there are an increasing number of cows milked in an AMS which includes different milking routines. Therefore we tested two different automatic milking systems milking routines influence on milk ejection and milk removal. Pre-milking teat preparation for 1 min is sufficient to induce OT release and milk ejection before the start of milking in both systems for all cows. In well filled udders, 30 s of pre-milking teat preparation is sufficient (i.e. early lactation or longer milking interval).

In conclusion, the udders of mares, ewes and cows differ anatomically. However sufficient pre-milking teat preparation is needed to collect alveolar milk and reduce residual milk. AMS require individual pre-milking teat preparation, while in conventional milking systems this fact is less crucial. Mixed milking systems (milking and suckling) require longer pre-milking teat preparation for complete milk removal. Complete milk removal in ewes is more dependent on morphological characteristics after milk ejection occurs.

The knowledge gathered in this work is a useful tool to optimize pre-milking teat preparation requirement for the observed species and milking systems.

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Original publications (peer reviewed):

Dzidic A, Knopf L, Bruckmaier RM.
Oxytocin release and milk removal in machine-milked mares.
Milchwissenschaft, 2002, 57: 423-424

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20.-21.09.2001

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APPENDIX

Oxytocin release and milk removal in machine-milked mares.

Milchwissenschaft 57(8): 423-424.

Oxytocin release and milk removal in machine-milked mares

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In this work oxytocin release and milking characteristics during machine milking of mares were investigated. Two experiments were performed twice each in all animals. In experiment 1 mares were milked twice subsequently and residual milk was collected after milking 2 with injection of oxytocin (10 i.u. i.v.). In experiment 2 mares were also milked twice, but residual milk was collected already after milking 1. Oxytocin concentrations peaked during pre-stimulation and decreased after the teat cups were attached. Total milk yield was 1.1±0.1 kg during milking 1 and 0.5±0.1 kg during milking 2 in experiment 1. Residual milk obtained after second milking was 0.7±0.1 kg. Total milk yield during milking 1 in experiment 2 was also 1.0±0.1 kg. Residual milk obtained after milking 1 was 0.9±0.1 kg. As expected there was scarcely milk left in experiment 2 after the residual milk was collected. A short peak without plateau phase characterizes milk flow curves in both experiments. Obviously oxytocin release during machine milking was insufficient for complete udder emptying.

Oxytocinfreisetzung und Milchejektion beim Maschinenmelken von Stuten

In der vorliegenden Arbeit wurden die Ocytocinfreisetzung, Milchejektion und Milchflussverlauf beim Maschinenmelken von Stuten untersucht. Die Behandlungen 1 und 2 wurden bei allen Tieren jeweils zweimal durchgeführt. In Behandlung 1 wurden die Stuten zweimal gemolken, wobei nach der zweiten Melkung die Residualmilch durch Injektion von 10 I.E. Ocytocin gewonnen wurde. Auch in Behandlung 2 wurden die Tiere zweimal gemolken, die Residualmilch wurde allerdings bereits nach dem ersten Melken gewonnen. Während der Vorstimulation kam es zu deutlicher Ocytocinfreisetzung, bereits zum Melkbeginn nahmen die Ocytocinkonzentrationen jedoch wieder ab. Die Gemelksmenge war in Behandlung 1 bei der ersten Melkung 1.1±0.1 kg und bei der zweiten Melkung 0.5±0.1 kg. Nach der zweiten Melkung ergab sich noch eine Residualmilchmenge von 0.7±0.1 kg. In Behandlung 2 war die Gemelksmenge bei der ersten Melkung ebenfalls 1.0±0.1 kg. Die nach der ersten Melkung gewonnene Residualmilch war 0.9±0.1 kg. Erwartungsgemäß war bei der zweiten Melkung in Behandlung 2 nahezu keine Milch mehr zu gewinnen. Die Milchflussverläufe wiesen alle eine spitze Form auf ohne die Ausprägung einer Plateauphase. Offenbar reichte die Ocytocinfreisetzung während des Maschinenmelkens nicht aus, um eine völlige Euterentleerung zu gewährleisten.

04 Milk removal (mares, oxytocin removal)   **04 Milchentzug** (Stuten, Oxytocinfreisetzung)

1. Introduction

The importance of oxytocin (OT) release and milk ejection for complete milk removal during machine milking are well documented for cows, goats and sheep (1). In mares, high concentrations of exogenous OT were shown to support milk ejection and milk removal (2). The goal of this work is to investigate the OT release and milking characteristics during machine milking of mares. Additionally, mammary cistern size before and after milk ejection was measured by using ultrasound imaging.

2. Materials and methods

Three experimental mares of the breed Süddeutsches Kaltblut were kept at a commercial farm, and were routinely machine milked. Two mares were in their 4th lactation, while one mare was in lactation 7. Milking was performed after mares have been separated from their foals for about 5 h.

The udder cisterns were visualized by B-mode ultrasound imaging. Cross sectioning including gland and teat cavities was performed in water bath from below the teat in ventral direction (3), before and after milk ejection as induced by exogenous OT (10 i.u. i.v.).

During all experiments milk flow was continuously recorded using a strain gauge system with differentiation unit and strip chart recorder (4). Parameters evaluated were total milk yield (TMY), total milking time (tMT), average milk flow (AMF), peak flow rate (PFR) and residual milk (removed after injection of 10 i.u. OT i.v.). All results are presented as means ± SEM.

2.1 *Experimental protocols*

Two experimental protocols were performed twice each in all animals.

2.2.1 *Experiment 1*

Mares were milked after a 1-min pre-stimulation that consisted of cleaning of the udder with a wet towel, hand milking of the first strips and drying of the udder. The cluster was removed after cessation of milk flow and stripping. After a break of 15 min, the mares were milked again (milking 2). After the end of milking 2 OT (10 i.u.) was injected i.v. to remove residual milk. During Experiment 1 blood samples for determination of OT concentrations were taken from the jugular vein via a cannula (Braun Braunüle 4/G 12, effective length: 8 cm, D-34209 Melsungen, Germany) at -2 min, and -1 min (start of pre-stimulation) and from then on every 30 s throughout both milking. OT concentrations were determined by radioimmunoassay (5).

2.2.2 *Experiment 2*

The milking procedure was similar as in Experiment 1 except for removal of residual milk, which was performed already after the first milking. No blood samples were taken during Exp. 2. Milking 2 started after the break of 15 min from removal of residual milk.

3. Results and discussion

Mean total cistern cross section obtained from ultrasound was 18±1 cm². After OT application, a cistern area enlargement of 38±12% was observed. The OT base line level was 4.2±0.6 ng/l. Highest OT concentrations were observed during pre-stimulation in both milkings (Fig. 1). Our data suggest that OT release is necessary to obtain alveolar milk fraction in mares and probably more intensive prestimulation is needed in order to maintain elevated OT concentrations throughout milking. TMY was similar during the first milking in both experiments (Table 1). Similar results were found previously (2, 6). It seems likely that after the cistern fraction from the relatively small cistern was removed, there was only a part of the alveolar milk collected while the transient OT release was not sufficient to completely empty the udder. In experiment 2 the amount of residual milk was 87 % of the TMY obtained in milking 1 (Table 1). Furthermore, TMY in milking 2 was very low. The amount of milk, as well as milking characteristics during milking 2 suggest there was no alveolar milk left over from previous milking after removal of residual milk. It can be assumed that the OT release was followed by milk ejection as shown in Fig. 2. Milk flow curve is characterized by very high PFR of only about 1 min duration.

4. Conclusions

OT release followed by milk ejection is necessary for collection of the alveolar milk in mares. Obviously a transient OT release occurs which is mainly present during the pre-stimulation phase. Milk ejection during subsequent machine milking remained incomplete.

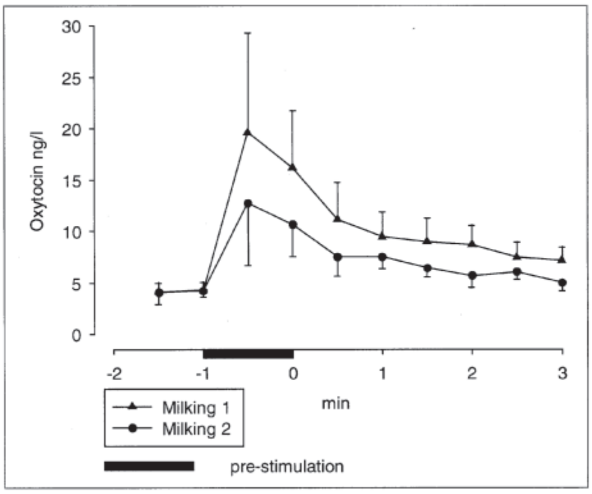

Fig. 1: Mean OT profiles during milking 1 and milking 2

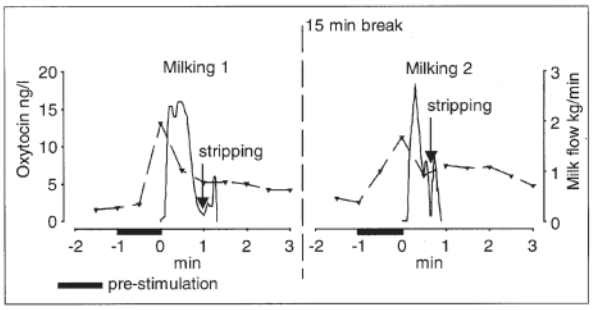

Fig. 2: Milk flow rates and corresponding OT concentrations of an individual mare during experiment 1

Table 1: Milking characteristics in experiment 1 and 2

Milking characteristic	Experiment 1 Milking 1	Experiment 1 Milking 2	Experiment 2 Milking 1	Experiment 2 Milking 2
Milk yield, kg	1.09±0.08	0.45±0.07	1.02±0.14	0.03±0.02
Milking time, s	154±7.71	117±10.4	137±13.8	25±1.76
Average milk flow, kg/min	0.44±0.04	0.25±0.04	0.44±0.04	0.08±0.06
Peak flow rate, kg/min	2.44±0.22	1.85±0.27	2.89±0.36	1.18±0.49
Residual milk, kg	–	0.66±0.08	0.89±0.07	–

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Oxytocin release, milk ejection and milking characteristics in a single stall automatic milking system

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Oxytocin release, milk ejection and milking characteristics in a single stall automatic milking system

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Abstract

The objective of this study was to evaluate the effect of teat cleaning by two rolling brushes on oxytocin (OT) release, milk ejection and milking characteristics during milking in a single stall automatic milking system (AMS). Five treatments B0 (no brushing), B1 (one brushing cycle for 16 s, 4 s per teat), B2, B4 and B6 (two, four and six brushing cycles, respectively) were randomly changed and quarter milk flow curves were recorded. In addition, blood samples were taken from 10 randomly selected cows during milking at 1-min intervals for OT determination in treatments B0, B2, B4 and B6. Basal OT concentrations were similar (2.7 to 3.9 pg/ml) in all treatments. At the start of milking, OT concentration was lower ($P < 0.05$) in B0 as compared to all other treatments. At 1 min after the start of milking and throughout milking OT concentrations did not differ between treatments. Time until occurrence of main milk flow was lower in well filled udders as compared to udders with small amounts of milk stored. Frequency of the bimodal curves is decreasing with increasing number of brushing cycles. The teat cleaning device in the used AMS was suitable to induce OT release and milk ejection before the start of milking in treatments B2, B4 and B6.

Keywords: Oxytocin release; Milk ejection; Milking interval; Automatic milking system

1. Introduction

Tactile stimulation of the mammary gland causes through a neuro-endocrine reflex arc milk ejection. Milking routines in automatic milking systems (AMS) are different from conventional milking. Cows are voluntarily milked throughout the day at variable milking intervals. Teat cup attachment requires usually more time than in conventional milking (Hopster et al., 2000; Macuhova et al., 2003). There are four different devices of teat cleaning present in AMS: (1) Sequential cleaning by brushes or rollers, (2) simultaneous cleaning by a horizontal rotating brush, (3) simultaneous cleaning in the same teatcups as used for milking and (4) sequential cleaning of individual teats by a separate cleaning device (De Koning et al., 2002). Brushing of teats and udder for 60 s and teat cup attachment without continuous pulsation in multibox AMS (Macuhova et al., 2003) induce oxytocin (OT) release and milk ejection. Pre-milking teat cleaning for 75 s in a single stall AMS positively influenced milk ejection (Bruckmaier et al., 2001). In early lactation or after long milking interval more

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cisternal milk is available than in late lactation or after short milking intervals (Knight et al., 1994; Pfeilsticker et al., 1996). During milking after short milking intervals, i.e., at low udder filling and in late lactation, the cisternal fraction is small or missing and additionally alveolar milk ejection is prolonged (Bruckmaier and Hilger, 2001). Milking on empty teats can occur at the start of milking in case of too short pre-stimulation. Longer milking intervals were shown to increase milk flow rate and decrease milk production per hour (Hogeveen et al., 2001). The objective of this study was to evaluate the effect of the duration of teat cleaning by two rolling brushes on OT release, milk ejection and milking characteristics during milking in a single stall AMS.

2. Materials and methods

2.1. Animals

Forty-five German Fleckvieh cows were investigated during their voluntary milkings throughout three experimental weeks. Animals were in their first to sixth lactation. Animals were designated to three lactational stages: early (<100 days), mid (100–200 days) and late (>200 days) lactation. All cows were routinely milked in a single stall AMS (Merlin, Lemmer-Fullwood). Cows that were not entering the AMS voluntarily (milking interval longer than 12 h) were driven to the milking robot twice daily. The AMS computer selected cows visiting the AMS for milking based on their expected milk production (≥6 kg in days 0–250 and ≥5 kg in days >250, of lactation, respectively). All cows received concentrate during milking depending on their production level (at least 200 g/cow per milking and up to 6300 g/cow per day). Cows were fed a total mixed ration on the feeding lane.

2.2. Experimental design

Five treatments, B0 (no brushing), B1 (one brushing cycle), B2 (two brushing cycles), B4 (four brushing cycles) and B6 (six brushing cycles) were randomly assigned to 45 cows using cross-over design. Each brushing cycle lasted for 16 s, i.e., 4 s per teat. The five treatments were randomly changed from day to day (from 05:00 until 24:00 h the following day). On five additional days, blood samples were taken during milking from 10 randomly selected cows through a permanent catheter which was inserted in the jugular vein the day before the start of experiment. Samples were taken before udder brushing and from start of brushing until the end of milking at 1-min intervals in treatments B2, B4 and B6. In treatment B0 sampling started before the start 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 radioimmunoassay (Schams, 1983). Quarter milk flow was recorded during all experimental milkings with an especially rebuilt set of four Lactocorders (WMB, Balgach, Switzerland) as described (Wellnitz et al., 1999).

2.3. Data evaluation and statistical analyses

Data are presented as means ± standard error of the means (S.E.M.). Descriptive data for milking interval and udder filling were calculated for all milkings, except when the attachment process totally failed and the cow was released from the AMS without being milked. Milkings with successful teat cup attachment during first attachment attempt, no reattachment during milking, no incomplete milking and no technical failure caused by the AMS were used for evaluation of OT concentration and milking characteristics. Milking time was defined as the time from the first teat cup attachment until the last teat cup was removed. Degree of udder filling was estimated as a percentage of actual milk yield compared to maximum storage capacity. Maximum storage capacity of the mammary gland was estimated as the highest milk yield obtained at one successful milking (with milking interval not longer than 12 h) in month 2 of the respective lactation. Filling classes (FC) were defined as a grouped percentage of the degree of udder filling: 0–20% (FC 1), 20.1–40% (FC 2), 40.1–60% (FC 3), 60.1–80% (FC 4) and 80.1–100% (FC 5). Bimodality was detected when any of the quarter milk flow curves had a flow pattern with two increments separated by a clear drop of milk flow below 200 g/min shortly after the start of milking. The time until main milk flow was calculated in bimodal curves as the time from the start of milking until start of the second milk flow increment (Bruckmaier and Blum, 1996), while other curves (non-

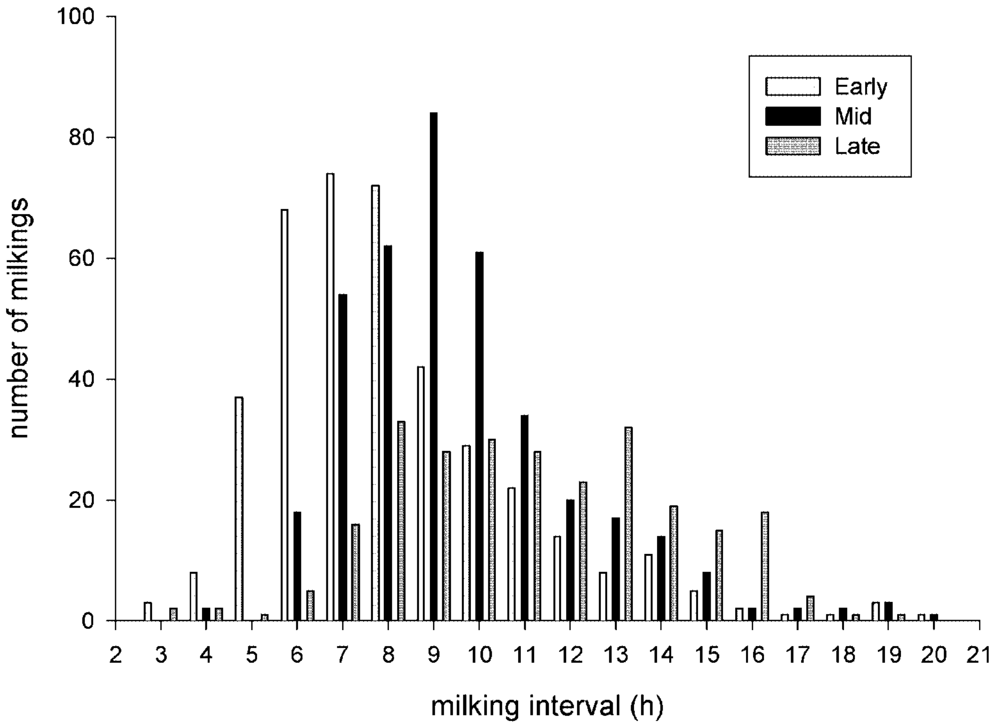

Fig. 1. Distribution of the intervals between milkings in early (n = 401), mid (n = 384) and late (n = 258) lactation during the experimental period.

bimodal and bimodal without cisternal milk fraction) as the time from the start of milking until the start of first milk flow increment. For milking characteristics, time until start of main milk flow and OT data evaluation, analyses of variance was calculated using the MIXED procedure of SAS (SAS 8.01, 1999). In the repeated measurement model udder filling, treatment and treatment × FC were defined as fixed effect, and animal nested within treatment was defined as a random effect. Pairwise differences between treatment means were tested using the Tukey–Kramer test with multiple comparison adjustment. Number of bimodalities was evaluated using logistic regression function with binomial distribution and logit link function. The effects in this model were the same as in the linear models for the other variables. Differences between treatments were tested for significance ($P < 0.05$) using the Wald chi-square test.

3. Results

3.1. Milking intervals and udder filling

Milkings were evenly distributed throughout the day. One percent of recorded milkings was with total

Table 1
Least square means of oxytocin concentrations in treatments B0, B2, B4 and B6

Oxytocin (ng/l)	B0	B2	B4	B6
Premilking baseline	3.1±0.3	2.7±0.4	3.9±0.4	3.0±0.3
End of brushing	3.3±4.5[a]	16.3±4.7[b]	17.6±4.6[b]	26.3±4.4[b]
1 min of milking	27.1±6.1	23.5±6.2	20.5±6.3	27.1±6.1
Entire milking AUC/min	27.1±4.4	25.5±4.5	20.2±4.5	20.6±4.4

[a,b] Least square means with different superscripts differ (P<0.05).
B0 (no brushing), B2 (two brushing cycles), B4 (four brushing cycles) and B6 (six brushing cycles).

failure of the attachment process. Mean milking interval was 9.4 h for all cows, which corresponds to the daily average milking frequency of 2.6. Two percent of all milking intervals were longer than 16 h and 5% shorter than 6 h. Percentage of milkings (n=1043) in each FC were 0, 12, 49, 32 and 6% in FC 1, FC 2, FC 3, FC 4 and FC 5, respectively. Most of the milkings occurred in FC 3 and FC 4 throughout lactation (77, 86 and 81% in early, mid and late lactation, respectively). Mean udder filling rate was 54±1, 57±1 and 57±1% in early, mid and late lactation, respectively. First lactation cows udder filling was 52±1, 61±1 and 55±1%, while in older cows was 56±1, 55±1 and 62±2% in early, mid and late lactation, respectively. The milking interval increased throughout lactation and was 8.2±0.1 h in early, 9.6±0.1 h in mid and 11.1±0.2 in late lactation, respectively (Fig. 1).

3.2. Oxytocin

Pre-milking baseline OT concentrations were similarly low in all treatments (Table 1). At the end of brushing OT concentrations increased in B2, B4 and B6, while OT remained low in B0 (Table 1). However, 1 min after the start of milking OT concentrations in B0 was similarly increased compared to the other treatments and area under curve (AUC)/min of

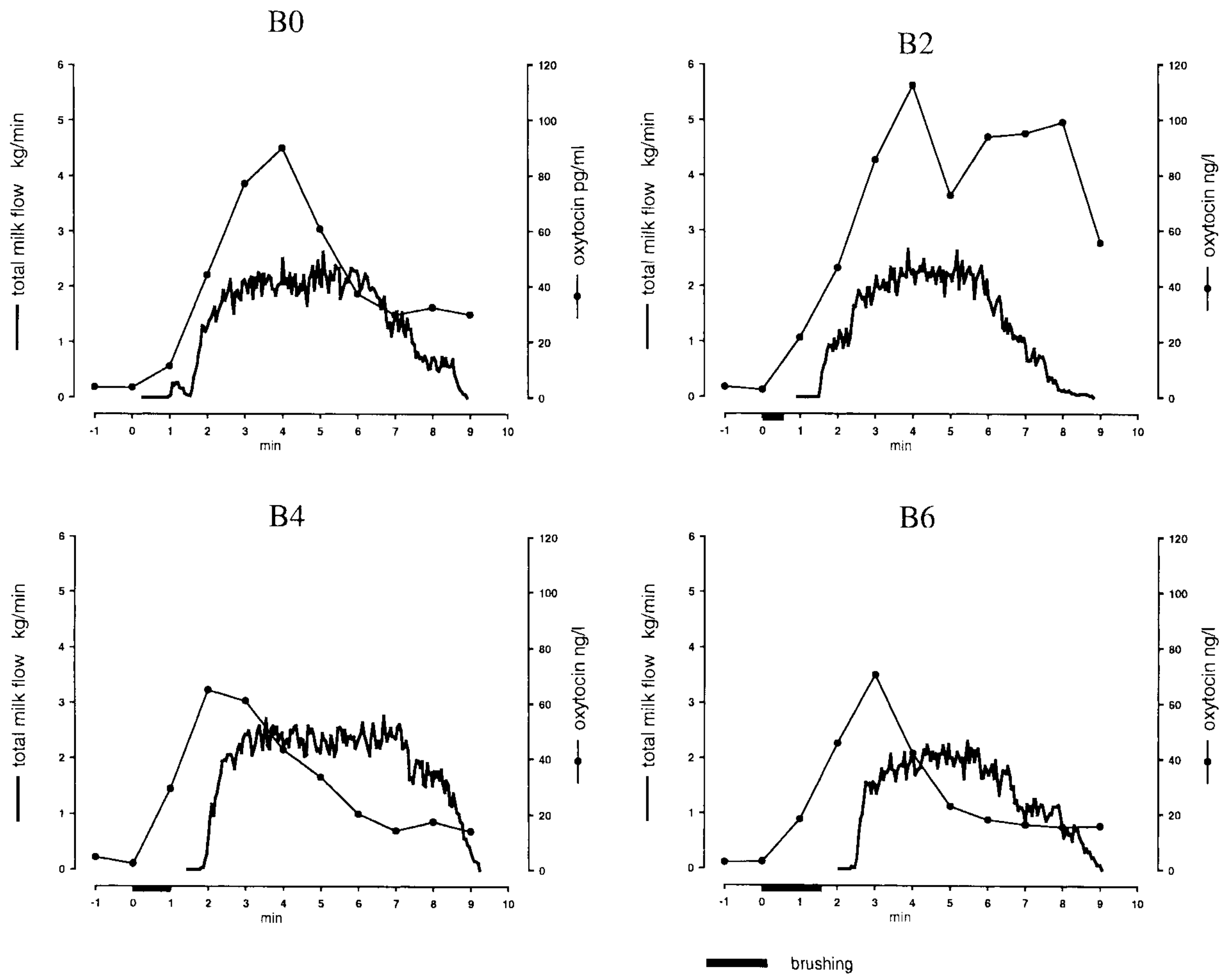

Fig. 2. Oxytocin concentrations and total milk flow of a representative animal in different treatments B0 (no brushing), B2 (two brushing cycles), B4 (four brushing cycles) and B6 (six brushing cycles).

OT throughout milking did not differ between treatments. All OT profiles increased during and after brushing and remained elevated throughout milking (Fig. 2).

3.3. Milking characteristics

With no (B0) or one (B1) cycle of pre-milking teat preparation, time until start of main milk flow was significantly shorter ($P<0.05$) in more filled udders compared to less filled udders (Table 2). In contrast, within treatments B2, B4 and B6 no differences of time until start of main milk flow were observed for different FC. Time until start of main milk flow was shortest ($P<0.05$) with longer duration of pre-milking teat preparation (B4 and B6) in 20–60% filled udders. In udders containing 60–80 and 80–100% of maximum filling the shortest time ($P<0.05$) until start of main milk flow was already observed with two and one cleaning cycles, respectively. More time was needed until main milk flow occurred in less filled udders then in more filled udders when the same pre-milking teat preparation was applied. Within the same udder filling class, more brushing cycles applied resulted in sooner occurrence of the main milk flow. Time from start of brushing until first attached teat cup was 42.7±0.9, 57.4±0.7, 89.6±1.0, and 121.4±0.4 s in B1, B2, B4 and B6, respectively). Time needed from end of brushing until attachment of the first teat cup without previously touching the teat was 24.6±0.4 s. As soon as a teat cup was attached milking for this particular quarter started. Time from first until last attached teat cup was 20.1±0.4 s. The portion of bimodal curves within a treatment decreased ($P<0.05$) with increasing number of brushing cycles (38, 21, 8, 0 and 0% in B0, B1, B2, B4 and B6, respectively). Milk yield and milking time as well as quarter milk yield and quarter milking time did not differ between treatments, although milking time as well as all quarter milking times were numerically longest in treatment B0. Milking time was shorter in front (4.5±0.1 min) than in rear quarters (5.7±0.1 min). Time from start of cleaning until the end of milking was 6.73±0.2, 6.58±0.2 and 6.68±0.2 min with 0, 1 and 2 cleaning cycles, respectively, i.e., 1 and 2 cleaning cycles somewhat reduced the total time required for milking. Treatment × FC interaction on milking time tended to be significant ($P=0.06$). Cows with more filled udder (FC 4 and FC 5) with the same pre-milking teat preparation applied needed more time to complete the milking process compared to cows with less filled udders (FC 2 and FC 3). Average flow rate was not significantly different between treatments. However, there was a significant ($P<0.05$) treatment × FC interaction on average flow rate. Within treatment higher flow rates corresponded with higher FC. Average flow rate was lowest ($P<0.05$) for cows with 20–40% of udder filling, when no and one cycle of pre-milking teat preparation was applied. Exceptionally, average flow rate was significantly higher ($P<0.05$) in the class 80–100% of filling (FC 5) compared to the other filling classes within the treatment with longest duration of pre-milking teat preparation (B6). However, the number of observa-

Table 2
Least square means of time until main milk flow in all treatments at different degree of udder filling

Time until main milk flow in treatments (s)	Udder filling (%)							
	20.1–40		40.1–60		60.1–80		80.1–100	
B0	74 ± 3^{Aa}	(15)	65 ± 2^{Aa}	(48)	54 ± 2^{Ab}	(40)	44 ± 4^{Ab}	(11)
B1	60 ± 5^{ABa}	(6)	45 ± 2^{Ba}	(51)	35 ± 2^{Bb}	(45)	32 ± 5^{ABb}	(5)
B2	38 ± 5^{B}	(6)	34 ± 2^{C}	(69)	27 ± 2^{BC}	(34)	21 ± 4^{B}	(11)
B4	25 ± 4^{C}	(12)	22 ± 2^{D}	(56)	20 ± 2^{C}	(47)	19 ± 2^{B}	(8)
B6	20 ± 2^{C}	(7)	21 ± 2^{D}	(62)	19 ± 2^{C}	(42)	15 ± 6^{B}	(5)

a,b Least square means without common superscript within row are significantly different ($P<0.05$).
A,B,C,D Least square means without common superscript within column are significantly different ($P<0.05$).
n, Number of observations.
B0 (no brushing), B2 (two brushing cycles), B4 (four brushing cycles) and B6 (six brushing cycles).

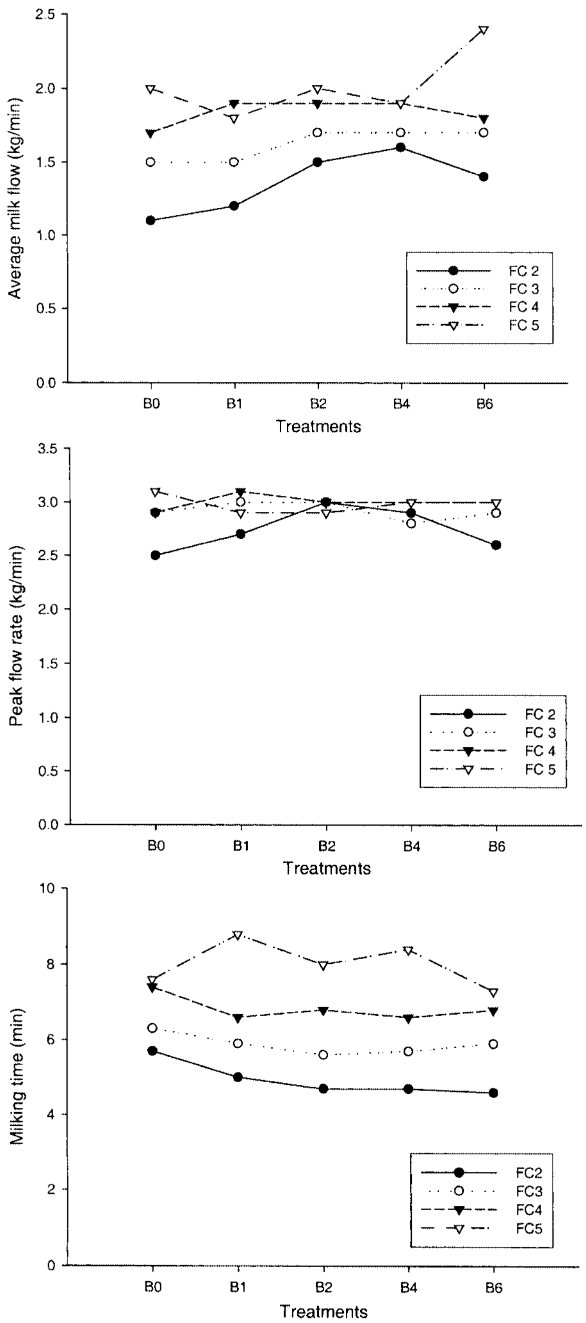

Fig. 3. Milking characteristics at different treatments B0 (no brushing), B2 (two brushing cycles), B4 (four brushing cycles) and B6 (six brushing cycles) throughout different filling classes FC 2 (udder filling rate 20.1–40%), FC 3 (udder filling rate 40.1–60%), FC 4 (udder filling rate 60.1–80%) and FC 5 (udder filling rate 80.1–100%).

tions in FC 5 within treatment B6 was very small (only five) and four out of five recorded cows had average flow rate higher than 2.1 (kg/min). Peak flow rate did not differ between the treatments. The effect of the interaction treatment × FC on peak flow rate was also not significant ($P = 0.12$), although within treatment B0 lowest FC 2 showed a significantly ($P < 0.05$) lower peak flow rate compared to all other filling classes (Fig. 3). Udder filling rate was almost unchanged throughout the treatments (from 58 ± 1 to 59 ± 1%), as well as milking intervals (from 8.9 ± 0.2 to 9.3 ± 0.3 h).

4. Discussion

Degree of udder filling at cows' voluntary visits to the AMS was constant, most cows visited the AMS in FC 3 and FC 4, while minimum milking interval was changed with a cow's production level throughout lactation. Nevertheless, 19% of the milkings occurred at very high (FC 5) or low (FC 2) udder filling. Furthermore, optimisation of the cows' visits to the AMS caused a prolongation of the milking interval with proceeding stage of lactation (Weiss et al., 2002), resulting in an average milking interval of more than 11 h in late lactation. Nevertheless, 85% of milkings occurred at milking intervals shorter than 12 h.

Pre-milking baseline OT concentrations were similarly low in all treatments, i.e., no conditioned release of OT before tactile stimulation did occur (Bruckmaier and Blum, 1996, 1998). Without teat cleaning (no tactile stimulation) OT concentrations remained at the baseline level. Our data show that both the rotating brush during teat cleaning and the teat cup liner during milking are able to induce a sufficient OT release for milk ejection within 1 min from the start of stimulation (Bruckmaier and Blum, 1996). Obviously, timing of OT release rather than absolute OT concentration is crucial for optimal milk removal (Schams et al., 1984; Bruckmaier et al., 1994; Bruckmaier and Hilger, 2001). The time until the start of main milk flow was shown to decrease with increasing udder filling in treatments B0 and B1. In treatment B2 there was no further significant decrease in time until start of main milk flow, as compared to B1. Therefore, two cleaning cycles are certainly sufficient in well filled udders, while less filled udders require four cleaning cycles.

Probably, minimum time until start of main milk flow (15–27 s) corresponds with the time when alveolar milk ejection occurred. A part of this time may be needed for forestripping, milk transport from the cluster through the pipes to the Lactocorder and overcoming the teat sphincter barrier if milk is already present in cisternal area. As expected, the time until alveoli contract when they are partially filled takes more time as compared to completely filled alveoli (Bruckmaier and Hilger, 2001). In late lactation or after short milking interval lag time from start of pre-stimulation until milk ejection increases (Bruckmaier and Hilger, 2001). In early lactation the milking cluster can be attached earlier while the amount of cisternal milk is larger (Pfeilsticker et al., 1996; Rasmussen et al., 1992). When time after the start of milking until main milk flow is delayed without or with a very small cisternal fraction, empty milking is the consequence. Over-milking causes ringing of the base of the teat which remains unrelieved during the longer part of the milking time (Hillerton et al., 2002). This could cause further inhibition of the milk let-down and tend to reduce milk yield (Wellnitz et al., 1999). If pre-milking preparation time is added to the attachment time in treatments B2, B4 and B6 on average 60, 90 and 120 s, respectively, are needed until main milk flow occurred. This delay (1–2 min) from start of stimulation until onset of milk ejection was already shown to increase with decreasing udder filling or in late lactation (Bruckmaier et al., 1994; Bruckmaier and Hilger, 2001; Mayer et al., 1991). Time from start of attachment until all four teat cups were attached was on average 45 s in our experiment. A similar attachment time (66 s) with the same attachment principle was found by Hopster et al. (2000). Comparing all the attachment principles Ipema (1996) reported 0.6 min as optimal attachment time of all four teat cups. Although attachment time is longer than in conventional system it does not influence OT release and milk ejection, because already the first teat cup attached provides adequate teat stimulation (Bruckmaier et al., 2001). Short attachment delay after pre-stimulation did not cause any negative effect on milking characteristics in conventional (Rasmussen et al., 1992) and robotic milking routines (Bruckmaier et al., 2001). Milking time showed a tendency to be increased without stimulation prior to milking as already shown in conventional milking systems (Mayer et al., 1984; Zinn et al., 1982). In our experiment, an optimal reduction of total milking time (milking time including pre-milking teat preparation time) was found for treatments B1 and B2 as compared to B0. Average flow rate showed a similar tendency to increase with increasing time spent on pre-milking teat preparation (Rasmussen et al., 1992), while lowest values were observed with B0 and B1 within FC 2. The highest average flow rate ($P < 0.05$) was calculated in treatment B6 in FC 5. Obviously this is an effect of the small number of observations and only the cows with higher milk flow rates. The data from this study clearly show that treatment B4 is sufficient for all the cows in experiment concerning the OT release, milk ejection and milking characteristics. Further optimisation of the pre-milking teat preparation could be done by adjusting the permission for milking to the actual udder filling. It is obvious that a more filled udder need less pre-milking teat preparation in order to avoid milking on empty teats and at the same time to reduce time needed for pre-stimulation, i.e., more efficient AMS.

5. Conclusion

The teat cleaning device in the used AMS was suitable to induce milk ejection before the start of milking in treatments B2, B4 and B6. At low degree of udder filling (i.e., after short interval from previous milking or late lactation) cows needed a longer pre-stimulation for well-timed induction of milk ejection. Optimal milk removal in cows with less udder filling (FC 2 and FC 3) was observed after four cleaning cycles (i.e., a pre-stimulation time of 64 s), while in more filled udder (FC 4 and FC 5) already with two cleaning cycles. Higher efficiency of the AMS could be obtained with adjustment of the threshold for cow acceptance for milking based on the actual degree of udder filling. Prolonged milking intervals towards the end of lactation compensate for reduced production rate causing unchanged udder filling rate during AMS milkings.

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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 (AMS) were studied in two experiments on Red Holstein/German Fleckvieh crossbreed cows. Milking routines used were milking: (1) Without premilking teat preparation; (2) With one cleaning cycle (58-60 s) with cold (13-15ºC) water; (3) With one cleaning cycle with warm water (30-32ºC); or (4) With two cleaning cycles (122 s) with warm water. In experiment 1 milking characteristics were evaluated and milking routines were randomly assigned to 62 cows during three measuring periods of 24 h each. In experiment 2 ten randomly selected cows were assigned to the same milking routines during four days 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. Pre-milking preparation with cold compared to warm water showed no differences in OT release, milk yield, peak flow rate, main milking time, average flow rate and time until main milk flow. Baseline OT concentrations were consistently low. At the start of teat cup attachment without pre-milking teat preparation OT concentrations remained on the basal level but were elevated in all other treatments. Already 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 in the used AMS, similarly 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 pre-milking teat preparation is performed to ensure clean teats and complete milk ejection before milking is started. Pre-milking teat preparation in automatic milking systems (**AMS**) differs from that in conventional milking. Four different principles of the pre-milking teat preparation are available in AMS (De Koning et al., 2002). Pre-milking teat preparation with simultaneous brushing of all four 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). Pre-milking teat preparation with cold (10ºC) compared to warm water (40 or 46ºC) showed no differences in milking characteristics during conventional milking (Dodd and Foot, 1947; Frommholdt, 1973). Only in a study in Jersey cows a prolonged stimulation was required if cold water was used (Phillips, 1984). AMS milking is performed at variable milking intervals. Commencement of milk ejection is delayed in late lactation or after a short interval from previous milking (Bruckmaier and Hilger 2001). Short milking intervals in AMS increased milk production rate of high producing cows, while milk flow rate was lower as compared to 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 pre-milking teat cleaning influences OT release and milk removal during milking in AMS. Pre-milking 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/year) were investigated during their voluntary milkings in experiments 1 and 2. Cows were designated to three lactational stages: early (<100 days), mid (100 to 200 days) and late (>200 days) lactation. All cows were routinely milked in AMS (VMS, DeLaval, Tumba, Sweden) with guided cow traffic (cows can only reach the feeding area by passing the AMS: Harms et al., 2002). 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 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 performed by blowing air between the liner and the teat during taking off the teat cup. One cleaning cycle consisted of cleaning of all four teats 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, while cows producing more than 22 kg/d received 1.5 kg/d of concentrate. All animals were kept indoor, and were ad libitum fed a mixed roughage ration (65% maize silage and 35% grass silage) at the forage lane. In the stable cows were offered concentrate from two automatic feeders depending on their production levels (at least 0.5 kg/cow/d and up to 10.5 kg/cow/d).

Experiment 1

Sixty-two cows in their first to fifth lactation were randomly assigned to four treatments in crossover design during three recording periods over 8 mo. Each recording period lasted for 4 d. Treatments were randomly changed every 24 h. Treatments of pre-milking teat preparation were no pre-milking teat preparation (T0), one cleaning cycle with warm water (30-32ºC; standard routine; T1), and cold water (13-15ºC; T1C), and two cleaning cycles with warm water (30-32ºC; T2). Quarter milk flow was recorded during all experimental milkings with an especially rebuilt set of four 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, one in its third and one 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, four in mid lactation, 161 ± 5 DIM, with an average milk production of 1.91 ± 0.1 kg/h, and four 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 catheterization day and four additional experimental days. Habituation of the cows to the experimental settings was performed 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 during one week prior to the start of experiment. 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 pre-milking teat preparation and the first two minutes 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 x g for 15 min. Blood plasma was stored at –20ºC until OT concentrations were determined by radioimmunoassay (Schams, 1983). Quarter milk flow was recorded as in experiment 1.

Data analyses

Data of milkings with unsuccessful attachment within one min from the start of teat cup attachment, reattachment of the teat cups during milking, incomplete milking (quarter milk yield less than 50% from expected quarter milk yield at 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 two increments separated by a clear drop of 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 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 first teat cup and removal of the last teat cup. Total milking time was defined as main milking time including pre-milking teat preparation.

In experiment 1, the following model was used:

$$Y_{ijklm} = \mu + T_i + L_j + D_k + I_l + Cow_m{*}T_i(L_j) + I_l^2 + T_i{*}D_k + T_i{*}I_l + T_i{*}I_l^2 + T_i{*}D_k{*}I_l + e_{ijklm} \qquad [1]$$

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),

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),

$Cow_m * T_i (L_j)$ = random effect of cow (m = 1 to 62) and treatment interaction within lactation class (error term for testing treatment effects),

e_{ijkl} = residual error.

In experiment 2, the following model was used:

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

where

Y_{ij} = OT values,

μ = overall mean,

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

$Cow_j (T_i)$ = random effect of cow (j = 1 to 10) within treatment (error term for testing treatment effects),

e_{ij} = residual error.

Pairwise differences between treatment means were tested by using 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 dataset 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 pre-milking teat preparation after 22 ± 1 s. Time needed to attach the first teat cup, with or without pre-milking teat preparation, ranged from 13 to 15 s (Table 1). Time to attach all four teat cups ranged from 53 to 56 s. Preparation lag time in treatments T1, T1C and T2 was 82, 82 and 145 s, respectively. Main milking time was significantly longer ($P < 0.05$) without pre-milking teat preparation as compared to all other treatments (Table 2). Quarter milking time without pre-milking 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, total and quarter milk yields did not differ between the treatments. Average flow rate was significantly lower ($P < 0.05$) without pre-milking 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 previous milking in treatment without pre-milking teat preparation as compared to all other treatments (Figure 1). Time until start of alveolar milk ejection without pre-milking teat preparation was decreasing up to 11 hours from previous milking ($P < 0.05$) (Figure 2). Numerically shortest time until occurrence of alveolar milk ejection without pre-milking 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 pre-milking teat preparation. Time until alveolar milk ejection without pre-milking teat preparation was shorter after long (68 s) than after short (102 s) milking interval.

Experiment 2

OT concentrations were measured during 69 milkings in 10 cows. OT 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. OT concentrations rose markedly within only 30 s after teat cup attachment started without pre-milking teat preparation. Peak OT concentrations were observed during pre-milking teat preparation in treatments T1, T1C and T2, and at 30 s of milking in treatment T0. OT 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. Pre-milking 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 pre-milking 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 minutes more residual milk and lower overall milk yields occur. A delay of 2 min after the end of a 1 min pre-milking 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 pre-milking teat preparation and attachment of the first teat cup (i.e. start of milking) is more crucial for milking performance than the duration of pre-milking teat preparation. If pre-milking 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 pre-stimulation is applied, total milking time (main milking time including pre-milking teat preparation time) is usually not reduced (Rothenanger et al., 1995). In this study main milking time was longer in treatment without pre-milking teat preparation than in other treatments, most evident in the rear quarters. One or two cleaning cycles of pre-milking teat preparation compared to no pre-milking 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 since 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 pre-milking teat preparation. It seems likely that after 8 h from 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 delayed occurrence of alveolar milk ejection at milking without pre-milking 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 (T0: start of teat cup attachment). Peak concentration lasted for one to two minutes and thereafter decreased gradually, while remaining above baseline until the end of milking. OT release during milking (AUC/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). Pre-milking teat preparation of one or two cleaning cycles prevented delayed milk ejection and no bimodal milk flow curves, indicating delayed milk ejection occurred. Milking without pre-milking 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 40ºC) compared to cold water (10ºC) did not influence milking characteristics during pre-milking teat preparation (Dodd and Foot, 1947; Frommholdt, 1973). On the contrary, Philips (1984) found significantly longer pre-milking teat stimulus requirement with cold (15ºC) than with hot water (54ºC). A part of this difference could be assigned to the postulated longer pre-milking stimulus required by Jersey as compared to 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, while 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 pre-milking 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 in the used 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.

Milking routine with one or two cleaning cycles (i.e. pre-milking teat preparation of 58 to 60 s and 122 s) induced OT release and milk ejection independently from the milking interval or stage of lactation. Alveolar milk ejection occurs at about one minute from start of teat stimulation by liner after longer milking interval, or up to two minutes after short milking interval. Adequate pre-milking teat preparation time should be applied to the cows at milking intervals shorter than 8 h.

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Table 1. Descriptive statistics of pre-milking teat preparation phases in **AMS**

Trait	T0[1]		T1[2]		T1C[3]		T2[4]	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Time to attach 1st teat cup (s)	13.1	0.4	13.9	0.4	14.5	0.5	13.3	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

[1] T0 - treatment without pre-milking teat preparation.

[2] T1 - one cycle of teat cleaning with warm water (30-32ºC), standard routine.

[3] T1C - one cycle of teat cleaning with cold water (13-15ºC).

[4] T2- two cleaning cycles with warm water (30-32ºC).

Table 2. Milking characteristics in different pre-milking teat preparation in Experiment 1. Least square means ± SEM.

Milking characteristic	Treatment				SEM	I[5]	T x 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	***	*

[1] T0 - treatment without pre-milking teat preparation.

[2] T1 - one cycle of teat cleaning with warm water (30-32ºC), standard routine.

[3] T1C - one cycle of teat cleaning with cold water (13-15ºC).

[4] T2- two cleaning cycles with warm water (30-32ºC).

[5] I – milking interval

[a,b] Least square means with different superscripts differ (P < 0.05).

*P< 0.05.

***P < 0.001.

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

[1] AUC – area under the curve.

[2] T0 - treatment without pre-milking teat preparation (n = 15).

[3] T1 - one cycle of teat cleaning with warm water (30-32ºC), standard routine (n = 18).

[4] T1C - one cycle of teat cleaning with cold water (13-15ºC) (n = 20).

[5] T2- two cleaning cycles with warm water (30-32ºC) (n = 16).

[a,b] Least square means with different superscripts differ ($P < 0.05$).

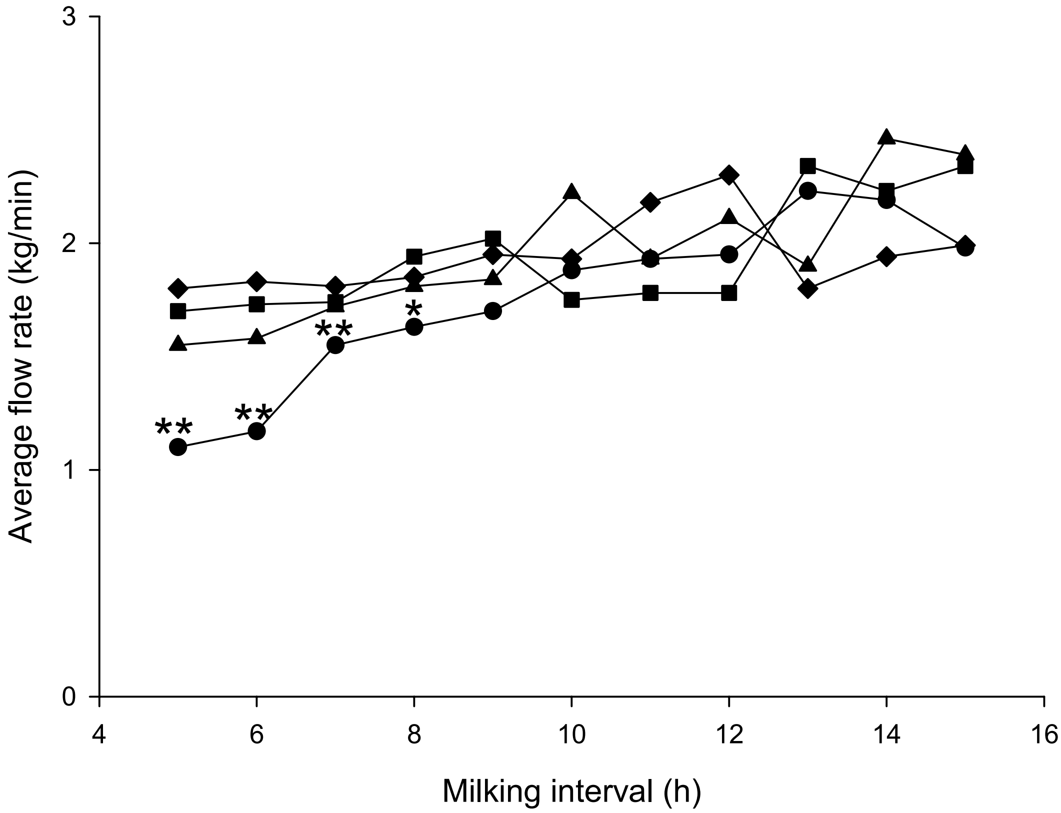
Figure legends.

Figure 1. Mean average flow rate in different milking intervals for treatment without pre-milking teat preparation (T0, ●), or one cycle of teat cleaning with warm water (30-32ºC), normal routine (T1, ♦), or one cycle of teat cleaning with cold water (13-15ºC) (T1C, ▲), or two cleaning cycles with warm water (30-32ºC) (T2, ■). Two asterisks indicate differences ($P < 0.01$) and one asterisk ($P < 0.05$) between the treatments.

Figure 2. Time until alveolar milk ejection in bimodal curves without pre-milking teat preparation. Means ± SEM. Means without common superscript are significantly different ($P < 0.05$).

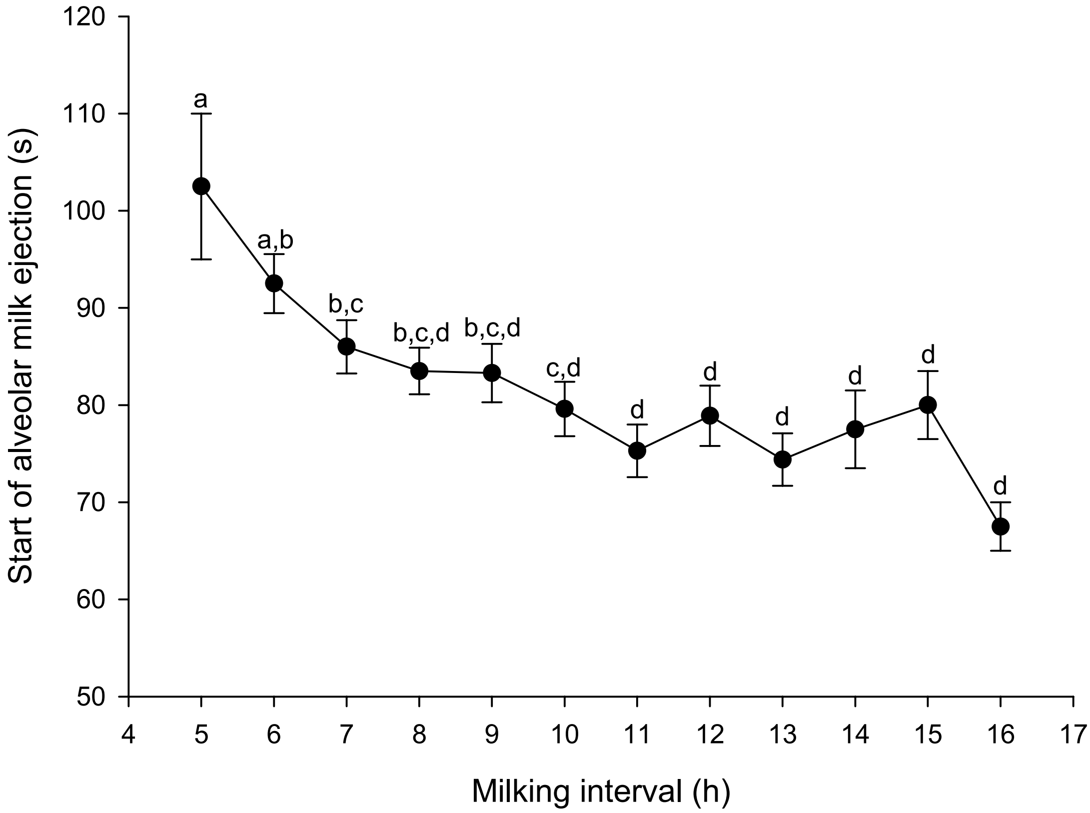
Figure 3. Oxytocin profiles, release during milking, total and quarter milk flow in one cow. (T0) Milking routine without pre-milking teat preparation. (T1) Milking routine with one cycle of teat cleaning with warm water (>30ºC). (T1C) Milking routine with one cycle of teat cleaning with cold water (<15ºC). (T2) Milking routine with two cleaning cycles with warm water (>30ºC). Teat cup or first teat cup (↑) attachment and teat cup or last teat cup (↓) removal in quarter and total milk flow curve. Duration (▬) of pre-milking teat preparation.

FIGURE 1.



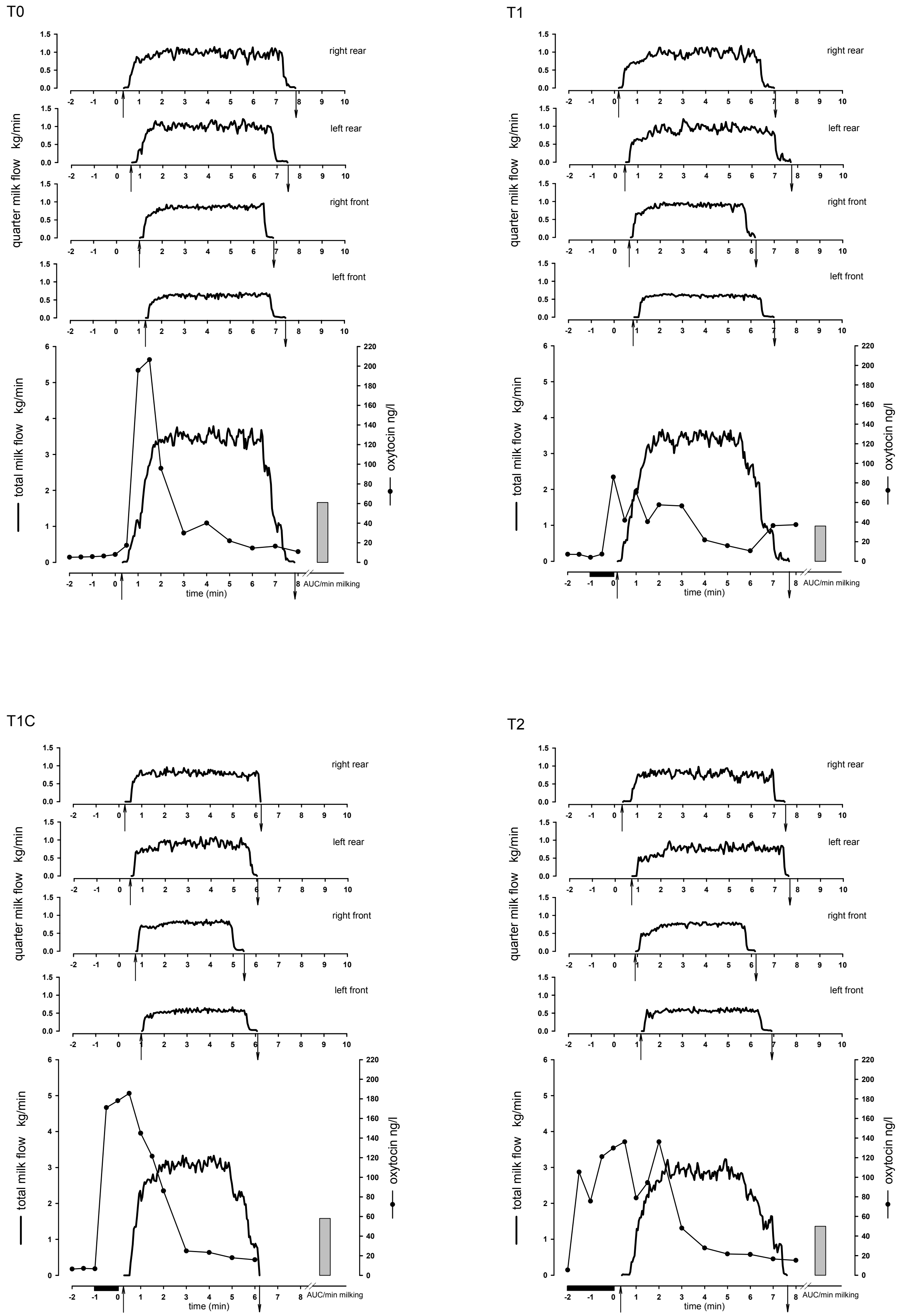
Average flow rate (kg/min)
0
1
2
3
4
6
8
10
12
14
16
Milking interval (h)
**
**
**
*

Figure 2.



Start of alveolar milk ejection (s)
120
110
100
90
80
70
60
50
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Milking interval (h)
a
a,b
b,c
b,c,d
b,c,d
c,d
d
d
d
d
d
d

Figure 3.



T0
T1
T1C
T2
right rear
left rear
right front
left front
quarter milk flow kg/min
total milk flow kg/min
oxytocin ng/l
time (min)
AUC/min milking

Machine milking of Istrian dairy crossbreed ewes: udder morphology and milking characteristics

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Small Ruminant Research, in press

Abstract

The objective of this study was to evaluate the differences in udder morphology and milking characteristics between Istrian dairy crossbreeds. A total of sixty three Istrian dairy crossbreed ewes (twelve crosses with 75% Istrian and 25% Awassi, IAI; fourteen crosses with 25% Istrian, 25% Awassi and 50% East Friesian, IAEF; and thirty-seven crosses with 50% Istrian and 50% Awassi, IA) were used in the experiment. Ewes were milked twice daily up to day 149 of lactation. Thereafter, they were milked once a day until the end of lactation (150 – 250 d). Udder morphology was evaluated in mid-lactation (90 – 120 d). The traits measured were udder volume, teat length, and teat angle. Milk flow recordings by using an especially calibrated mobile system for ewe milking (Lactocorder) were performed in early (month 2), mid (month 3 - 4) and late (month 6 - 7) lactation. Overall milk yield was 292 ± 10 kg during 203 ± 4 d of lactation in all crosses. Average daily yield was 1.4 ± 0.1, 1.4 ± 0.1 and 1.6 ± 0.1 kg/d for IAI, IA and IAEF crosses, respectively. Milk flow curves with one peak were mainly present in IAEF crosses, while IAI and IA crosses had an equal distribution of milk flow curves with one and two clearly separated peaks. Milk flow curves with diffuse shape and peak flow rate below 0.4 kg/min were equally low in all animals (8 to 11 %). Udder traits in all animals were 1.2 ± 0.1 l, 3.3 ± 0.1 cm, and 46 ± 1 deg for udder volume, teat length, and teat angle, respectively. Milking characteristics were 1.7 ± 0.4 min, 0.6 ± 0.2 kg, 0.7 ± 0.2 kg/min and 0.4 ± 0.1 kg/min for milking time, milk yield, peak flow rate and average flow rate, respectively. Milking characteristics were mostly positively correlated ($P < 0.001$), except for milking time and average flow rate ($r = -0.30$, $P < 0.05$). Moreover, udder volume was positively and significantly ($P < 0.001$) correlated with all milking characteristics. Peak flow rate tended ($P = 0.08$) to be higher in IAEF compared to IAI crosses. Udder volume increased with lactation number. Istrian dairy ewe crosses are well adapted to machine milking. The milk production tended to be higher in IAEF crosses compared to IA and IAI crosses.

Keywords: Dairy ewes, Udder traits, Milking characteristics

1. Introduction

Milking characteristics and udder morphology are one of the factors determining milkability in dairy ewes. Knowledge of milk yield, milking time and udder conformation is necessary for optimal adaptation of the milking environment to the needs of the ewe.

Milkability can be evaluated by analysis of the milk flow curves (Labussiere, 1988; Mayer et al., 1989; Peris et al., 1995; Bruckmaier et al., 1997; Marie et al., 1999; Marnet et al., 1999), milking characteristics (Mayer et al., 1989; Bruckmaier et al., 1997; Marie et al., 1999; Marnet et al., 1999) and udder morphology measuraments (Labussiere, 1988; Fernandez et al., 1995; Rovai et al., 1999).

The cisternal milk fraction in most dairy sheep breeds is greater than 50% (Caja et al., 1999). Therefore, a large portion of milk is located within the cisternal cavities. At least part of the cisternal milk is located below the orifice of the teat canal and can be collected only during stripping (Bruckmaier and Blum, 1992; Bruckmaier et al., 1997). Increase in milk production usually determines a larger udder with unfavorable position of the teats (Rovai et al., 1999). An ideal dairy ewe should have a large cistern and vertical placement of the teats (Labussiere, 1988).

Better understanding of morphological variations and milking characteristics would identify those traits which are most appropriate for incorporation in the Croatian dairy ewe selection program.

In Croatia, one domestic sheep breed is the Istrian dairy breed. There are about 20000 dairy ewes in the Istrian region and about 1000 of them belong to the autochtonious Istrian dairy ewe breed. The domestic Istrian dairy breed has been crossed with Awassi and East Friesian rams in order to improve their milk production.

The aim of this study was to evaluate the milking characteristics of Istrian dairy crossbreed ewes and to test the differences between different crosses. Additionally, the relationship between udder morphology and milking characteristics was analyzed.

2. Materials and methods

2.1. Animals and milking parameters

The study was conducted on the commercial dairy sheep farm Radosevic in Brtonigla, Istria, Croatia. A total of sixty-three Istrian crossbreed dairy ewes were used in the experiment: twelve crosses with 75% Istrian and 25% Awassi, IAI; fourteen crosses with 25% Istrian, 25% Awassi and 50% East Freisian, IAEF; and thirty-seven crosses with 50% Istrian and 50% Awassi, IA. The ewes were in their first to ninth lactation. The animals were kept on pasture, changing their lots every three days. The ewes were kept with their lambs until day 29 after lambing. At milking time, animals were brought to the stable for two h. During milkings ewes received grain pellets ad libitum. Milking was performed twice daily at 8 a.m. and 4 p.m. on days 30-150 of lactation. After day 150 of lactation ewes were milked once daily until the end of lactation. The milking parlour was designed for forty animals at a time. The ewes were milked with 6 milking units (De Laval, Tumba, Sweden) at a milking vacuum of 37 kPa, pulsation rate 120 cycles/min and pulsation ratio 50:50. The milk was collected in buckets. Milking routine consisted of attachment of teat cups without previous touching of the udder and machine stripping which consisted of manual udder massage and pulling of the lowest part of the udder up in order to have teats at the lowest position when the milk flow dropped below 100 g/min, while teat cups remained attached.

2.2. Udder morphology and milk flow curves

Udder traits were measured once in mid-lactation (90 – 120 d of lactation). Udder measurements were taken by one technician once for each ewe at approximately 2 h before the evening milking. Teat length was measured on both sides from teat base until teat orifice. Teat angle was measured in degrees from vertical line (intramammary ligament) of the udder from a caudal view. The actual volume of the udder was estimated by dipping the udder into a water filled bucket and measuring water displacement as previously shown in goats (Bruckmaier et al., 1994). Milk flow was continously recorded by using an especially calibrated Lactocorder (WMB AG, Balgach, Switzerland) for ewe milking. Milk flow recordings were performed in early (month 2), mid (month 3 - 4) and late lactation (month 6 - 7) during three consecutive days during morning and afternoon milkings. Milking characteristics included milking time, milk yield, peak flow rate, and average flow rate. Milk flow curves were evaluated according to the number of peaks as proposed earlier (Bruckmaier et al., 1997) and as shown in Fig. 1.

Individual milk production per lactation was estimated by using the method proposed by Thomas et al. (1999), which summed up milk production on the test day and multiplied it with the number of days between two test days.

2.3. Statistical analysis

For the milking traits the following repeatability model with lactation class and milking interval defined as blocks:

$y_{ijkmn} = \mu + CB_i + P_j + \delta_{ij} + \sum_m \beta_m (DIM^m{}_k) + I_n + \varepsilon_{ijkmn}$

where

y_{ijkmn} = measures of milk yield (kg), peak flow rate (kg/min), milking time (min), average milk flow (kg/min) at days in milk (DIM)k.

μ = overall mean,

CB_i = effect of cross breed (i = 1 to 3, IA, IAI and IAEF),

P_j = effect of lactation class (j = 1 to 9),

δ_{ij} = random error between ewes within crossbreed and lactation class, the error term for testing the crossbreed effect.

I_n = effect of milking interval (n = 8, 16 or 24 hours),

$\sum_m \beta_m (DIM^m{}_k)$ = cubic polynomial function describing the effect of days in milk (DIM)

ε_{ijkmn} = random error between measurements within ewe

For the udder conformation traits the following model was used:

$y_{ij} = \mu + CB_i + P_j + \varepsilon_{ij}$

where

y_{ij} = measures of udder conformation. (udder volume (l), teat length (cm), teat angle (deg))

μ = overall mean,

CB_i = effect of cross breed (i = 1 to 3, IA, IAI and IAEF),

P_j = effect of lactation class (j = 1 to 9),

ε_{ij} = random error among ewe

Pairwise differences between crossbreed means were tested by using the Tukey -Kramer test with multiple comparison adjustment. The analyses were done by using the MIXED procedure of SAS for milking characteristics and GLM procedure of SAS for udder morphology (SAS Institute, 1999). The relationships between milking and udder conformation traits were analyzed by estimating correlations between measures adjusted to the other effects in the corresponding models. From the milking model it was the best linear unbiased prediction (BLUP) of δ_{ij} for each ewe. From the udder conformation model it was the residual effects prediction of ε_{ij} for each ewe. Thus, the correlation estimates between all traits were calculated based on single intrinsic values of each ewe estimated from the corresponding models. The Pearson's correlation analyses were performed by using the CORR procedure (SAS Institute, 1999).

3. Results

The estimated lactational milk yield for the experimental ewes was on average of 292 ± 10 kg during 203 ± 4 days of lactation. The average daily milk yield was 1.4 ± 0.1 kg/d, 1.4 ± 0.1 kg/d, and 1.6 ± 0.1 kg/d in IAI, IA, and IAEF crosses, respectively.

During the study 627 milk flow curves were evaluated. The number of milk flow curves with one peak, two clearly separated peaks and diffuse shape milk flow with peak flow rate below 0.4 kg/min (Fig. 1) was similar for Istrian x Awassi crosses regardless of different blood percentages (Table 1). For the IAEF crosses more ewes with one peak milk flow curves were observed as compared to other crosses (78 vs. 45%). Daily mean milk yield and average flow rate at experimental milkings tended to be lowest in IAI and maximal in IAEF crosses (Table 2). Milking time was numerically longest in IAI and the shortest in IA crosses although differences were not significant. Peak flow rate was highest in IAEF compared to IA and IAI crosses ($P = 0.08$). There were no differences between crosses in teat length, teat angle and udder volume (Table 2).

Positive and significant ($P < 0.001$) correlations were observed between all milking characteristics except between milking time and average flow rate ($r = -0.27$, $P < 0.05$) and milking time and peak flow rate ($r = -0.15$, $P = 0.24$). Teat length did not show any correlation neither with other milking nor morphological characteristics (Table 3). Positive and significant ($P < 0.001$) correlations were also observed between udder volume and all milking characteristics. Conversely, negative correlations were obtained between udder volume and teat angle ($P < 0.05$). Teat angle was also negatively and significantly ($P < 0.001$)

correlated with milk yield and milking time. The udder volume was significantly increasing ($P < 0.05$) from the first (0.9 ± 0.1 l) to the ninth (1.6 ± 0.2 l) lactation. Lowest values were observed in first and second lactation, while in the third lactation the udder volume was close to maximum (1.4 ± 0.1 l). Teat angle and teat length did not change between lactations.

4. Discussion

The IAEF crosses showed 8 and 9% higher average milk production per day as compared to the IA and IAI dairy crossbreeds, respectively. The crossing of Istian x Awassi ewes with East Friesian rams resulted in higher milk production. In dairy ewes Labussiere (1988) described two types of milk flow curves with one and two peaks. In the present study we observed three types of milk flow curves, similary as in a previous study (Bruckmaier et al., 1997). Our data for IAEF crosses are similar to those in East Friesian ewes (Mayer et al., 1989; Bruckmaier et al., 1997), with most of the milk flow profiles with one peak. The one peak milk flow curve could occur with and without alveolar milk ejection (Mayer et al., 1989; Bruckmaier et al., 1997). Therefore, the one peak milk flow curve without alveolar milk ejection removes only the cisternal fraction during milking, while late response to milking possibly causes oxytocin release after the end of milking (Bruckmaier et al., 1997). On contrary, fast response to milking stimulation cause alveolar milk ejection, which results in presence of alveolar milk in the cistern, before the cisternal milk was removed. It seems likely that because of increased milk production through selection, the alveolar milk ejection is masked within the first peak (Marnet et al., 1998; Marnet and McKusick, 2001). The IAEF crosses tended to have the highest milk flow, average flow and milk production. The milk flow curve with two clearly separated peaks was similarly present in IA and IAI crosses showing alveolar milk ejection after the cisternal milk was removed. The milk flow curve with one peak in IA and IAI crosses most probably represents more cisternal than alveolar milk. The milk flow curves with diffuse shape and peak flow rate below 0.4 kg/min represent extremly weak or totally absent oxytocin release (Bruckmaier et al., 1997). This type of milk flow was similarly low in all three crossbreeds and corresponds with lower peak flow rate. Moreover, similar results in few studies (Mayer et al., 1989; Bruckmaier et al., 1997) with East Friesian breed showed that the milk kinetics classification for the whole udder with three types of the milk flow curves could be usefull for selection.

Teat length was similar to Manchega dairy ewe 65 to 80 d in milk, measured at 8 h after a.m. milking and Lacaune dairy ewe 10, 30, 60 and 120 d in milk measured 2 h prior to p.m. milking (Labussière et al., 1981; Rovai et al., 1999). Labussière et al. (1981) showed no significant correlation between teat length and milk production or milk emission kinetics. These results were confirmed in this study. Furthermore, crosses with shortest teats (IAEF) tended to have higher average and peak flow rates. The larger udder volume is associated with unfavourable position of the teats for milking which could cause falling off the teat cups (Labussiere, 1988). The teat angle was similar to Lacaune dairy ewe breed obtained at 50 d in milk and measured 8 h after a.m. milking (Labussiere, 1988). Such et al. (1999) showed in Manchega ewes and Malher and Vrayala-Anesti (1994) in French Rouge de l'Ouest ewes better teat position for the machine milking than in more milk producing Lacaune ewes. Similar results were obtained in our study, where less milk producing crosses (IA and IAI) had better teat position than more milk producing crosses (IAEF). Therefore, instead of only selection for udder volume and milk production the teat angle could be included into selection of Istrian dairy crossbreed ewes.

The positive and significant correlation between milk yield and peak flow rate suggest that high producing animals have fast milk removal, as shown in Lacaune ewes (Marie et al., 1999; Marnet et al., 1999). Moreover, fast milk removal reduces the milking time and increases parlour efficiency.

In the present study an increment of the udder volume with increased lactation class was observed. Similar increases of udder depth and heigth, i.e. udder volume, were found in Churra breed throughout lactation numbers (Fernández et al., 1995). The same study showed more horizontal teat position, i.e. reduced suitability of the udder for machine milking, in higher lactations (≥ 3) compared to first and second.

5. Conclusion

Although numerically the highest milk production as well as average and peak flow rate with good udder shape was obtained in IAEF Istrain dairy crossbreed, all three crossbreeds of Istrian dairy ewes are well adapted to machine milking. The udder volume influenced all milking characteristics positively, while the teat angle influenced milking time and milk yield negatively and showed no influence on flow rate. Since milk flow and udder morphology traits have influence on milking efficiency and indirectly on milk yield they should be considered in a breeding program for improving Istrian dairy sheep.

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Table 1

Frequency of milk flow curve types in Istrian dairy sheep crossbreeds

Crossbreed	Milk flow curve type, %		
	1	2	3
Istrian (50%) x Awassi (50%)	45	47	8
Istrian (75%) x Awassi (25%)	45	44	11
Istrian (25%) x Awassi (25%) x East Friesian (50%)	78	10	12

Milk flow curve type 1, milk flow with one peak

Milk flow curve type 2, milk flow with two peaks

Milk flow curve type 3, low milk flow with diffuse curve shape and peak flow rate < 0.4 (kg/min)

Table 2

Least square means of milking characteristics and udder morphology in Istrian dairy crossbreeds.

Trait	Crossbreed		
Milking characteristics	Istrian (50%) x Awassi (50%)	Istrian (75%) x Awassi (25%)	Istrian (25%) x Awassi (25%) x East Friesian (50%)
Daily milk yield, kg	0.58 ± 0.1	0.52 ± 0.1	0.75 ± 0.1
Milking time, min	1.68 ± 0.1	1.94 ± 0.1	1.79 ± 0.2
Peak flow rate, kg/min	0.64 ± 0.1[ab]	0.53 ± 0.1[b]	0.80 ± 0.1[a]
Average flow rate, kg/min	0.36 ± 0.02	0.29 ± 0.03	0.43 ± 0.05
Udder morphology			
Udder volume, l	1.27 ± 0.1	1.10 ± 0.1	1.51 ± 0.2
Teat angle, deg	46 ± 1	44 ± 2	49 ± 4
Teat length, cm	3.32 ± 0.1	3.36 ± 0.2	3.05 ± 0.3

[a,b] Means in a row with different superscript differ (P = 0.08)

Table 3

Correlation coefficients among udder measurements and milking characteristics in Istrian dairy crossbreed ewes (n = 63)

Traits[a]	MY	MT	PFR	AFR	UV	TL
MT	0.47***					
PFR	0.75***	-0.15				
AFR	0.68***	-0.30*	0.93***			
UV	0.79***	0.49***	0.53***	0.41***		
TL	0.01	-0.04	0.09	0.03	-0.03	
TA	-0.32*	-0.41***	-0.09	0.01	-0.27*	-0.04

[a] MY = milk yield, MT = milking time, PFR = Peak flow rate, AFR = average flow rate, UV = udder volume, TL = teat length, TA = teat angle.

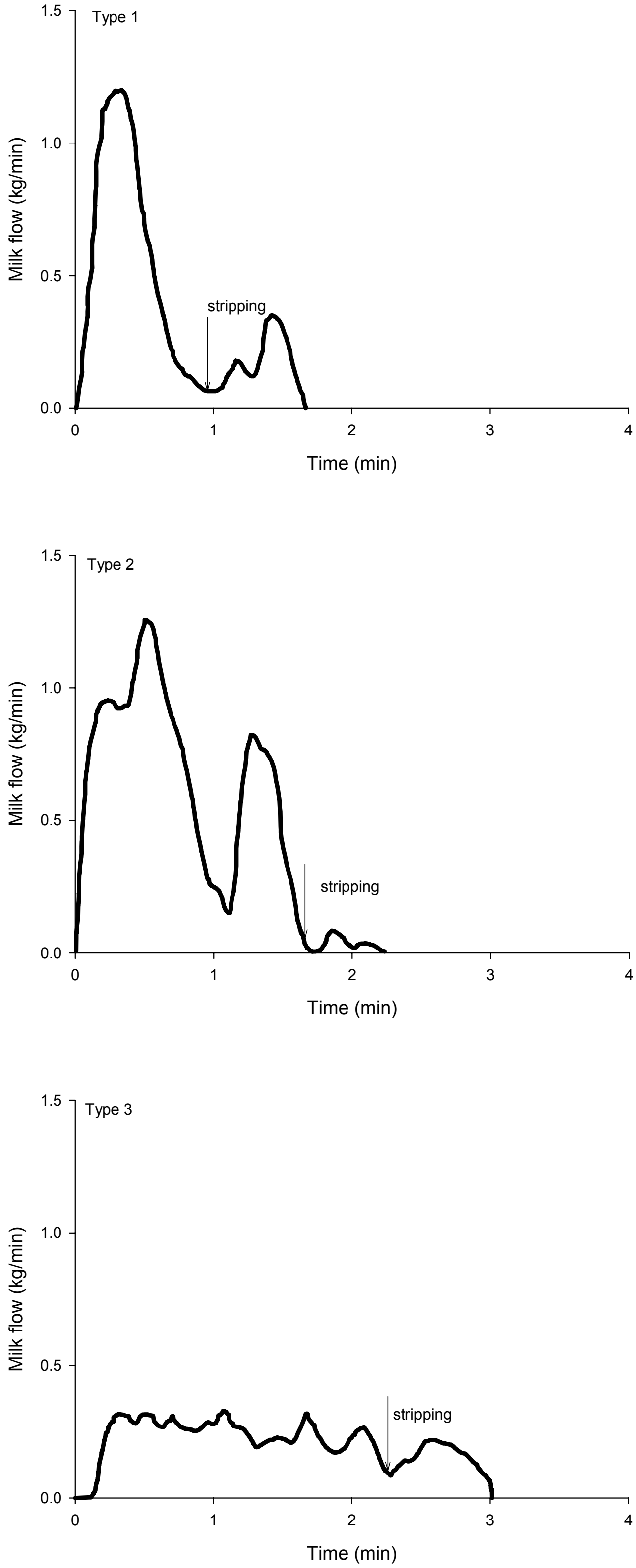
* $P < 0.05$

*** $P < 0.001$

Figure legends.

Fig. 1. Types of milk flow curves found in Istrian crossbreed dairy ewes (individual milkings). 0, start of milking; ↓ stripping. Type 1 and 2 curves are from Istrian dairy ewe crossbreeds (Istrian (50%) x Awassi (50%)), the type 3 curve from Istrian dairy ewe crossbreeds (Istrian (75%) x Awassi (25%)).

Fig. 1.


Type 1
Milk flow (kg/min)
1.5
1.0
0.5
0.0
stripping
0
1
2
3
4
Time (min)
Type 2
Milk flow (kg/min)
1.5
1.0
0.5
0.0
stripping
0
1
2
3
4
Time (min)
Type 3
Milk flow (kg/min)
1.5
1.0
0.5
0.0
stripping
0
1
2
3
4
Time (min)