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Swedish University of Agricultural Sciences
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Improvements in milking management through imitation of calf behaviour

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Kalvens beteende som modell för mjölkningsrutiner

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Abstract

Approximately 20% of the milk within the udder of a cow is stored in the cistern and immediately available for milk removal, while activation of the milk ejection reflex is required for removing the remaining milk stored in the alveolar compartment. The aim of this literature review is to describe, evaluate and consider implications of the three different suckling activities that the calf displays during a suckling bout. In terms of milking, these phases are represented by pre-stimulation, stimulation during milking and post-stimulation. Pre-stimulation is commonly performed in order to induce the milk ejection reflex, whereby the hormone oxytocin is released and the alveoli in the secretory tissue are contracted. Time requirements regarding pre-stimulation must be considered in relation to lactation stage and milking interval. The pre-stimulation should be sufficient to cause an alveolar contraction that is maintained until start of milking. During the milking phase, the liner within the teat cup should carry out a continuous stimulation and cause a maintained elevated concentration of oxytocin in the circulation during the entire milking. This will maximise milk yield. Furthermore an increased rate of massage during the final minute of milking has been found to increase milk and fat yield. Hence, development of liner settings within modern milking equipment and changes in the pulsation cycle may result in a significant improvement within dairy production.

Sammanfattning

Syftet med föreliggande litteraturstudie har varit att beskriva mjölkningsrutiner för kor utifrån de tre stimuleringsfaser som naturligt utgör en kalvs beteende under digivning. Dessa stimuleringsfaser är förstimulering, stimulering under mjölkning och efterstimulering. Förstimulering och stimulering under mjölkning förekommer i dagliga mjölkningsrutiner, men inte efterstimulering. Då endast cirka 20% av mjölken är lagrad i juvercisternen och direkt tillgänglig vid mjölkning, samtidigt som resterande mjölk är lagrad i alveolerna och kräver en inducering av mjölknedgivningsreflexen, är det viktigt att förstimulering tillämpas. Under hur lång tid förstimuleringen ska pågå tycks bero på laktationsstadiet och juverfyllnad. Förstimuleringen leder till frisättning av hormonet oxytocin, som i sin tur medverkar till att alveolerna i juvret kontraheras. Stimuleringen för nedgivning av mjölken sker under hela mjölkningen. Denna stimulering fås av spengummirörelserna, orsakade av tryckförändringar inne i spenkoppen, och leder till en bibehållen hög koncentration av oxytocin under hela mjölkningen. Gällande efterstimulering har tidigare försök påvisat en ökning i mjölmängd samt fetthalt då efterstimulering tillämpats. Således tycks det finnas ett behov av att utveckla spengummirörelsen så att mer massage induceras under sista minuten av mjölkning samt behov av att undersöka dess påverkan på mjölkavkastning och mjölksammansättning. Potentiellt kan detta leda till en ökad effektivitet inom mjölkproduktionen.

Introduction

Artificial milking has the same aim as naturally nursing a calf: to remove the milk from the udder for food supply. Knowledge of calf behaviour associated with suckling is hence of importance when designing milking procedures. During nursing, the calf tends to perform two types of behaviours: butting and suckling. As these behaviours are altered in frequency, the suckling bout is divided into three main phases. First

there is pre-stimulation consisting of frequent butting that induces milk ejection (Lidfors et al., 1994; Mayntz & Costa, 1998). The pre-stimulation is followed by a phase of milk intake where the calf applies vacuum and positive pressure to extract milk (Lidfors et al., 1994; Rasmussen & Mayntz, 1998). Finally, the bout ends with a post-stimulation phase where both suckling and butting are altered (Lidfors et al., 1994; Mayntz & Costa, 1998).

The question is whether the three suckling phases in calf nursing are important to consider in milking routines. Stimulation of the udder is carried out by the calf during the whole suckling bout and tends to be more intense before and after the actual milk removal (Lidfors et al., 1994; Mayntz & Costa, 1998). In milking routines when collecting milk for human consumption, cleaning of the udder resembles the pre-stimulation (Bruckmaier & Blum, 1996; Weiss et al., 2003) and the milking machine carries out a stimulation of the teats during milking (Mein, 1992; Bruckmaier et al., 1994b). There is however no routine that resembles post-stimulation. As post-stimulation is performed by the calf even as it grows older, it likely plays a role in nursing through stimulating further milk production in the mammary gland (Fjäderhane, 1992; Lidfors et al., 1994; Haley et al., 1998). The aim of this literature review is to discuss whether it during machine milking is important to consider the three different suckling activities displayed by the calf during nursing.

Pre-stimulation

Milk ejection and stimulation

In the teat skin, there are touch-sensitive receptors. Tactile stimulation of these receptors by a calf, a hand or a milking machine will induce a neuroendocrine reflex and release of the hormone oxytocin from the hypothalamus (Ely & Peterson, 1941; Gorewit et al., 1992). Tactile stimulation before milking is referred to as pre-stimulation and should be carried out to induce a milk ejection (Mayer et al., 1991; Bruckmaier & Blum, 1998). There is an approximately 30-60 seconds interval between pre-stimulation and an increased concentration of oxytocin in the blood (Mayer et al., 1986). As the increased concentration of oxytocin reaches a threshold level, it will induce a contraction of the myoepithelial cells surrounding the alveoli (Linzell, 1955). In this way, milk is forced from the alveoli to the cistern.

Without the occurrence of a milk ejection, only cisternal milk is available when milking, assuming that the teat sphincter barrier is overcome (Knight et al., 1994). It is thereby crucial to induce a proper milk ejection in order to have an efficient milk removal from the alveoli (Pfeilsticker et al., 1996). Beside tactile stimulation, visual and auditory stimuli have also been found to induce a release of oxytocin (Peeters et al., 1973 cited through Svennersten-Sjaunja, 2004; Pollock & Hurnik, 1978) as well as stimulation of the vagus nerve through feeding (Uvnäs-Moberg et al., 1985; Samuelsson et al., 1993; Johansson et al., 1999). Feeding during milking has also been found to have an impact on milk composition and udder emptying (Samuelsson et al., 1993; Johansson et al., 1999). Although, Bruckmaier and Blum (1998) state that the effect of conditional stimuli on the release of oxytocin is minor compared to tactile stimulation and should hence be seen as addition to teat stimulation.

Beside oxytocin, the hormones prolactin, vasopressin, growth hormone (GH) and cortisol are released during milking (Gorewit et al., 1992; Svennersten-Sjaunja &

Olsson, 2005). Oxytocin is the main hormone associated with the actual milk ejection during normal conditions, whereas both oxytocin and vasopressin have a significant role in the milk ejection for dehydrated ruminants (Mengistu, 2004). Prolactin and GH are involved in maintenance of lactation, where prolactin has a minor role in comparison to GH in dairy cows (Knight, 2001a). At the same time, prolactin and GH appear to be involved in the milk synthesis where prolactin is involved in protein synthesis and GH in fat synthesis (Knight, 2001b). Cortisol does not appear to have a role in milk synthesis but does have an effect on the metabolism of the animal as well as on the secretory activity of the epithelial cells (Sjaastad et al., 2010). However, this literature review focuses on oxytocin since that is the main hormone discussed in relation to milk ejection and stimulation.

Bearing in mind the important role of oxytocin in milk ejection it is pertinent to understand the mechanism behind oxytocin release. In order to obtain a milk ejection oxytocin must exceed a threshold level (Linzell, 1955). In cases where pre-stimulation is excluded in the milking routine, the result may be a bimodal milk flow curve. This is depicted in figure 1 where the blue line shows milk flow. A bimodal milk flow curve is characterised by delayed availability of alveolar milk after the removal of the cisternal milk fraction resulting in milk flow being close or equal to zero (Bruckmaier et al., 1994a; Dzidic et al., 2004). This hold up in milk flow is a result of late oxytocin release in relation to the time of cluster attachment and may cause mammary duct collapses and an increased risk of lesions as well as bacterial infections (Bruckmaier et al., 1994a; Bruckmaier & Blum, 1996; Dzidic et al., 2004). Performing any pre-stimulation that massages the teats before milking can therefore be said to be of vital importance in order to decrease machine-on time and increase milk flow rates (Phillips, 1984; Bruckmaier & Blum, 1996).

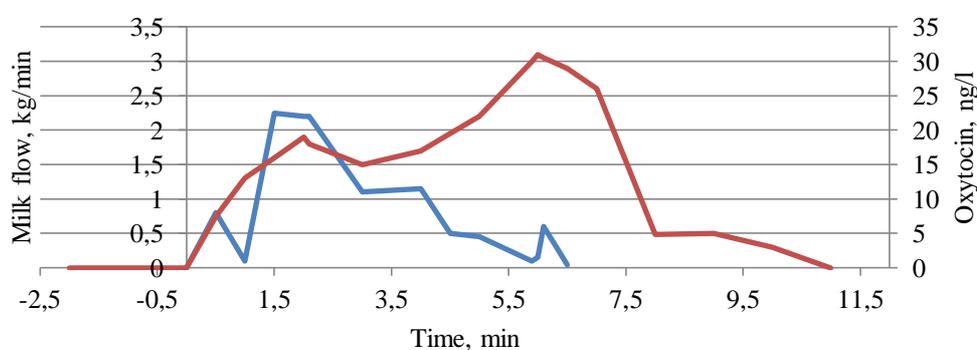


Figure 1. Milk flow (blue line) and oxytocin concentration (red line) during milking without manual pre-stimulation (modified from Bruckmaier & Blum, 1996).

Inhibition of milk ejection

There are two types of inhibition of the milk ejection: central and peripheral inhibition (Bruckmaier et al., 1997). Central inhibition is defined as inhibition of oxytocin release from the pituitary gland and may occur during emotional stress such as milking in unfamiliar surroundings (Bruckmaier et al., 1993; Bruckmaier & Wellnitz, 2008) or when cows are being switched from suckling to machine milking (Tančin et al., 1995; Tančin et al., 2001). Central inhibition results in a complete inhibition of the milk ejection or milk cessation before the udder is empty (Bruckmaier et al., 1997). A way of overcoming the problem associated with central inhibition is to use exogenous

oxytocin and thereby induce a milk ejection (Bruckmaier, 2003). However, it has been shown that long term use, i.e. beyond one week, of exogenous injections reduces natural milk ejection and therefore caution must be taken with such use.

Peripheral inhibition occurs when oxytocin is released but has no effect due to blockade of the oxytocin receptors (Gorewit & Aromando, 1985; Bruckmaier et al., 1997). Injection of atosiban, an oxytocin receptor blocking agent, before pre-stimulation results in complete inhibition of milk ejection and injection after pre-stimulation causes milk cessation (Bruckmaier et al., 1997). However, peripheral inhibition does only happen after exogenous injections of any oxytocin receptor blocking agent.

Pre-stimulation in different production systems

Milking in industrial countries is commonly performed through manual milking cluster attachment as for parlour milking, or within automatic milking systems (AMS). In countries where milk from cows is shared between human and offspring throughout lactation, systems of restricted suckling are more common (Orihuela, 1990; Krohn, 2001; Fröberg et al., 2007). The milking process carried out by the milking machine may be equal in different systems (Mein, 1992), but the phase of pre-stimulation is different.

When milking in conventional systems, pre-stimulation is performed through cleaning and massaging the teats as well as pre-milking before the milking unit is attached (Bruckmaier & Blum, 1996; Svennersten-Sjaunja, 2004). In the AMS, cleaning of the teats is mechanical and varies depending on type and brand. The different cleaning alternatives in the AMS are water or brush and both alternatives have been found to carry out proper pre-stimulation of the udder i.e. working as well as manual pre-stimulation (Bruckmaier et al., 2001; Mačuhová et al., 2003; Dzidic et al., 2004). Within the commission directive for the European Union, teat cleaning before milking must be performed due to hygienic reasons (EEC, 1989). This is however not a requirement in all parts of the world (Jargo et al., 2006; Davis et al., 2008).

Traditionally, in systems with restricted suckling a calf is used to perform the pre-stimulation and induce milk ejection through allowance to suckle before being tethered next to the cow (Orihuela, 1990). It has also been found that physical contact between the cow and calf without allowing the calf to suckle generates equal amount of sealable milk in dual-purpose farming (Combellas et al., 2003). Hence, there are restricted suckling systems where the calf is tethered next to the cow without being allowed to suckle until the end of the milking session as well (Combellas et al., 2003; Fröberg et al., 2007). However, no matter the specific routines in different restricted suckling systems, it is the calf that induces the milk ejection.

Pre-stimulation in relation to latency period

Pre-stimulation within conventional milking is commonly recommended to be performed during 30-60 seconds since this time has been found to be associated with an increased concentration of oxytocin in the circulation and result in non-bimodal milk flow curves (Sagi et al., 1980; Mayer et al., 1986). However, there is a great difference regarding continuity and stimulation time between conventional milking and AMS, especially when considering multi-box AMS with separate cleaning and

milking boxes (Bruckmaier et al., 2001). In conventional milking, there is commonly a continuous stimulation starting at the beginning of cleaning followed by the attachment of the milking unit. This statement does although assume that udder cleaning of no more than one cow is performed before the milking unit is attached. Within AMS, there is an interruption of the stimulation i.e. a latency period in between pre-stimulation and milking (Bruckmaier et al., 2001; Kaskous & Bruckmaier, 2011). Within systems of restricted suckling, the calf is commonly allowed to suckle for approximately 30 seconds before being tethered. A similar latency period as for the AMS is commonly also the case in restricted suckling as the tethering results in a pause in between pre-stimulation and milking.

There is a need for continuous alveolar contraction in between pre-stimulation and milking in order to completely empty the udder (Kaskous & Bruckmaier, 2011). A maintained alveolar contraction is commonly associated with the continuous stimulation in conventional milking (Bruckmaier et al., 2001). However, there is no negative effect on the oxytocin level and the alveolar contraction after a latency period shorter than 120 seconds after 60 seconds pre-stimulation. Moreover a short latency period of no longer than 45 seconds has been found to be required when applying a short pre-stimulation time of 15 seconds (Kaskous & Bruckmaier, 2011). Sequential teat cup attachment that may further prolong the latency period will not affect the alveolar contraction since stimulation of less than four teats induces a release of oxytocin that is sufficient for maintenance of alveolar contraction in all four udder quarters (Bruckmaier et al., 2001). What appears to be important is that milking routines are standardised and equal for every milking session so as to have cows being familiar with the milking routines (Rasmussen et al., 1990). Standardised routines of cows induce conditioned stimuli and increases milk yield. To summarise, there is a risk of cluster (unit) attachment before the milk ejection has started when excluding the latency period after a short pre-stimulation. Combining a short pre-stimulation time and a latency period is enough for inducing an oxytocin release with a maintained alveolar contraction until end of milking.

Pre-stimulation time in relation to udder filling and lactation stage

Automatic milking systems like AMS and automatic milking rotaries (AMR) have opened up a new area of research where it is possible to adjust milking to individual needs regarding pre-stimulation time. The cisternal fraction is commonly less than 20% and can sometimes be close to zero in systems with short milking intervals e.g. in AMS (Knight et al., 1994). A similar case can be seen in late lactation where there is a reduced cisternal milk yield and fraction due to decreased milk production (Pfeilsticker et al., 1996). In turn, the udder filling affects the pre-stimulation time needed in order to get a well-timed induction of milk ejection (Bruckmaier & Hilger, 2001; Dzidic et al., 2004). Udder filling does not depend on the amount (volume) of milk per se (Wellnitz et al., 1999). Instead, udder filling depends on storage capacity of the udder and the degree of alveolar compartment and/or cistern occupied by milk. A low udder filling resulting from a short milking interval requires a longer pre-stimulation time. Hence, there is no need of a long pre-stimulation at a high udder filling in order to get a milk ejection (Bruckmaier & Hilger, 2001; Weiss & Bruckmaier, 2005; Kaskous & Bruckmaier, 2011). The relationship between udder filling and seconds until milk ejection after the start of pre-stimulation is illustrated in figure 2, where the line is based on values obtained from different lactation stages (early, mid and late lactation) and different milking intervals (8 h and 12 h).

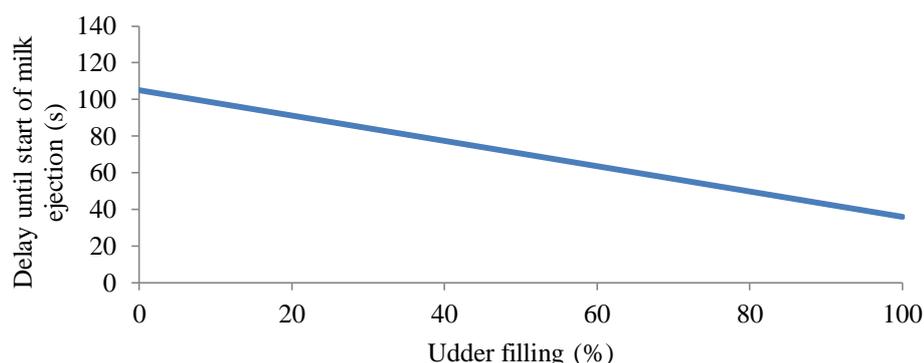


Figure 2. Relationship between delay until start of milk ejection (s) and udder filling (%) (modified from Bruckmaier & Hilger, 2001).

More contraction of the myoepithelial cells is needed at a low udder filling in order to result in milk ejection (Bruckmaier et al., 2001). The need of a prolonged pre-stimulation is thereby not an effect of reduced amount of oxytocin or a delayed release of the hormone (Mayer et al., 1991). Bruckmaier et al., (2001) explained the delayed milk ejection at low udder filling by describing a filled alveolus, high udder filling, with the shape of a ball and a partly filled alveolus with the shape of an ellipse (figure 3). In order to have an immediate release of milk, the ball shape is required. Therefore, a contraction of a filled alveolus results in an immediate ejection of milk. When the partly filled alveolus is contracted, it firstly takes the shape of ball and then ejects milk. The higher requirement of contraction results in more time needed until milk ejection.

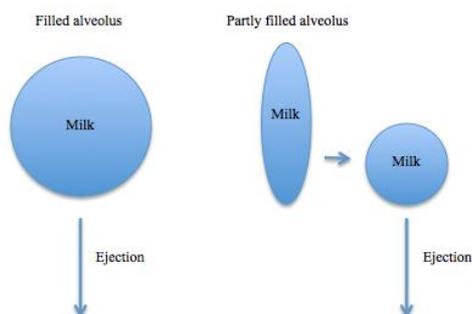


Figure 3. Illustration of milk ejection from a filled and a partly filled alveolus (modified from Bruckmaier et al., 2001).

Stimulation during entire milking process

Stimulation carried out by the milking machine

Suckling by a calf induces the highest release of oxytocin (Bar-Peled et al., 1995; Lupoli et al., 2001; Tančin et al., 2001). In turn, hand milking will generate a higher concentration of oxytocin throughout milking compared to stimulation from the milking machine (Gorewit et al., 1992). The milking unit of a milking machine works in a similar manner regardless of milking system. The technique applied by the calf

when drinking milk i.e. altering pressure and vacuum has been abandoned in modern milking machines due to technical difficulties (Rasmussen & Mayntz, 1998). Instead, milking machines only use vacuum to open up the teat canal (Mein, 1992).

The teatcups have a flexible inner sleeve called a liner that is opened and closed by cyclic changes in pressure and there are approximately 60 cycles per minute (Mein, 1992). During vacuum, the liner is opened and initiates milk flow. As the vacuum is later lost through regulation by the pulsator, the liner collapses, milk flow from the teats is prevented and milk is transported away from the milking machine. The function of the milking cluster is therefore highly dependent on the maintenance of vacuum inside the liner (Ambord & Bruckmaier, 2010). The milkability of a cow on udder level is determined by milkability at quarter level and may differ between quarters (Weiss et al., 2004).

The changes in pressure induce a pulsation that massages the teats (Mein, 1992). Both the massage carried out by the suckling calf (Rasmussen & Mayntz, 1998) and the stimulation performed by the liner of the milking machine, result in continuous release and maintenance of a high oxytocin concentration (Bruckmaier et al., 1994b). In turn, the high oxytocin concentration results in continuous contraction of the myoepithelial cells and efficient udder emptying.

Regarding stimulation carried out by the milking machine during milking, the massage phase of the pulsation cycle is important. The pulsation cycle is divided into four different phases; a, b, c and d (Mein, 1992), see figure 4. With regards to stimulation the d-phase is most interesting. This is the massage phase in the pulsation cycle during which the liner collapses and is kept closed. In order to induce a good stimulation and at the same time promote good udder health, the d-phase should be at least 150 milli seconds or contribute to at least 15% of the cycle (Svennersten-Sjaunja, 1995). During periods of high milk flow, the vacuum inside the milking tube decreases which shortens the d-phase (Svennersten-Sjaunja, 1995; Ambord & Bruckmaier, 2010). As a result, milk ejection may not be as well stimulated during the whole milking process (Bruckmaier & Blum, 1998) and the risk of oedema and congestion in the teat tissues increases (Mein, 1992).

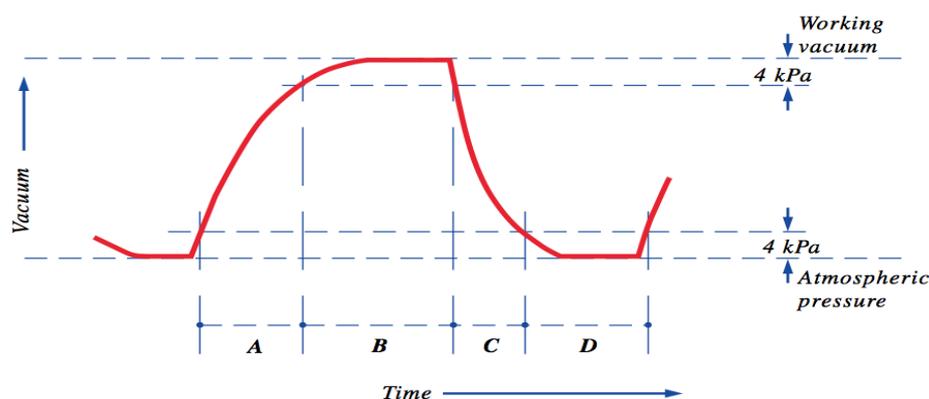


Figure 4. The pulsation cycle, a = opening phase, b = milking phase, c = closing phase, d = massage phase (from Svennersten-Sjaunja, 1995).

Stimulation during sequential teat cup detachment in AMS

In conventional milking, cluster detachment is often automatic and settings are made on udder level (Rasmussen, 1993). To compare, in the AMS teatcups are generally removed in each individual quarter at the end of milk flow (Bruckmaier et al., 2001). Hence, some quarters can still be milked and massaged while others are finished. The reduced stimulation of less than four teats does however not result in incomplete emptying of the teats being milked for longest time. Instead, stimulation of one teat at the end of milking is enough for causing an oxytocin release sufficient to result in complete milk ejection and udder emptying.

Post-stimulation

Research within the area of post-stimulation

Post-stimulation in the sense of increased massage during the final minute(s) of milking is not included in daily milking routines in conventional milking or in AMS. If it is partially included in restricted suckling systems depends on whether or not the calf is allowed to suckle after milking. Algers and Jensen (1985) were amongst the first to spark interest in this area, specifically looking at post-stimulation on pigs. They proposed the “restaurant hypothesis” i.e. an increased post-stimulation brought out by the individual piglet increases milk yield in the specific teat used (Algers & Jensen, 1985). They have also shown that piglets tend to be able to increase total milk yield produced by the sow through post suckling massage (Algers & Jensen, 1989).

There is only one study to be found that investigates the effect of implying post-stimulation in milking routines for cows (Fjäderhane, 1992). In this study, set in Sweden and carried out during 26 days in three periods, hand milking of one udder half was performed for the final minute of milking in order to induce extra massage. Results obtained indicate an increased amount of fat in milk from the post-stimulated quarter and an increased milk yield from both treated quarter and the remaining quarters that were machine milked simultaneously as treatment was carried out. These findings are similar to results obtained by applying extra stimulation of one quarter during the entire milking session (Svennersten, 1990; Svennersten et al., 1990; Svennersten & Barrefors, 1991).

Udder emptying and regulatory mechanisms

Despite findings suggesting that post-stimulation of the bovine udder may increase milk and fat yield, there is a lack of research in the area. Therefore, little is known that can explain the results found by Fjäderhane (1992). Post-stimulation likely results in an efficient udder emptying as an outcome of higher concentration of oxytocin associated with the increased massage (Lidfors et al., 1994), but may at the same time activate local regulatory mechanisms within the udder and thereby influence milk production within the mammary gland (Fjäderhane, 1992). The first findings regarding the action of local regulatory mechanisms within the udder were found after increasing the milking frequency on one udder half of goats. The treatment led to an increased milk yield in the same udder half (Henderson & Peaker, 1984). Similar results have also been found in cows (Allen et al., 1986; Stelwagen & Knight, 1997; Bar-Peled et al., 1998; Österman & Bertilsson, 2003). Likewise, a more complete udder emptying of an udder has a strong correlation with increased milk yield through

action brought out by local mechanisms (Wilde et al., 1987; Algers & Jensen, 1989; Peaker & Wilde, 1996).

One regulatory mechanism of interest associated with udder emptying and stimulation of milk production, is 5-hydroxytryptamin (5-HT) (serotonin). Serotonin is synthesised within the mammary gland from the amino acid L-tryptophan (Collier et al., 2012) and targets 5-HT receptors of which there are seven subfamilies (from 5-HT 1 to 7) within the mammary epithelium (Stull et al., 2007). The serotonergic system contributes towards a number of processes including the homeostatic regulation of mammary function in alveoli. In parallel with increased storage time of milk in the udder, there is an increase in serotonin within the interstitial fluid which activates 5-HT₇ receptors (Hernandez et al., 2008; Collier et al., 2012). Since 5-HT receptors are involved in reduction of the gene expression of α -lactalbumin and casein genes, the serotonergic system thereby inhibits milk secretion (Stull et al., 2007). In turn, the reduction of α -lactalbumin inhibits the production of lactose and further leads to a reduction in milk synthesis and yield (Hernandez et al., 2008). Serotonin also regulates transepithelial electrical resistance (TEER) within the udder and affects tight junction permeability (Nguyen & Neville, 1998; Stull et al., 2007). Tight junctions are located between the epithelial cells in the mammary gland and prevent leakage of milk into the extracellular fluid (Sjaastad et al., 2010). Not only does serotonin regulate TEER and affect tight junction permeability it also decreases the expression of two different tight junction protections involved in tight junction formation and maintenance (Marshall et al., 2009).

There is an on-going discussion regarding the similarities and differences between serotonin and another local regulatory mechanism called feedback inhibitor of lactation (FIL). FIL is thought to be a glycoprotein produced in the mammary secretory cells (Henderson & Peaker, 1984; Wilde et al., 1995). The concentration of FIL increases during milk stasis and FIL is removed together with the milk during milking. FIL is proposed to have a negative influence on the number and activity of secretory cells, and thereby explain the reduced milk yield when applying non-frequent and incomplete milking (Wilde et al., 1989). Both serotonin and FIL are synthesised in the epithelial cells of the mammary gland and they later affect the epithelial cells after being secreted in the milk (Wilde et al., 1995; Matsuda et al., 2004). They increase epithelial cell apoptosis, disrupt tight junctions and decrease milk protein (Matsuda et al., 2004; Collier et al., 2012). However, there appears to be differences between FIL and serotonin as well, including the location of the binding site and the mass weight of the molecule (Wilde et al., 1995; PubChemCompound, 2013). More research is needed in order to clearly state similarities and differences between the two compounds.

Discussion

Different social and environmental conditions, variation in herd sizes and availability of funds for investments lead to great differences in milk production systems around the world. Milk yield obtained during milking is however dependent on the release of oxytocin and the degree of udder emptying. In order to induce a well-timed milk ejection, pre-stimulation is needed before milking. Milk yield appears to be independent of the type of pre-stimulation (manual or mechanical) and regardless of whether or not there is a period of short-latency following pre-stimulation. This is

important since latency is common in both AMS and in practical conventional milking where teat preparation commonly is done on several cows before the milking unit is attached, despite literature describing conventional milking with continuous stimulation. Applying pre-stimulation increases udder health due to decreased risk of bimodal milk flow curves and sequential teat cup attachment as within the AMS does not affect milk yield in a negative way.

Factors that are important to consider when designing pre-stimulation routines are: standardised routines, avoiding the risk of inhibition of milk ejection (Bruckmaier et al., 1997; Bruckmaier & Wellnitz, 2008) and to accommodate individual requirements regarding pre-stimulation time (Weiss & Bruckmaier, 2005). Within conventional milking and systems with restricted suckling it is difficult to adjust pre-stimulation times to the individual. One suggestion to improve pre-stimulation in conventional milking is to sort cows into groups depending on lactation stage and not based on production level. Milking routines in the aspect of number of cows that are being prepared before the milking unit is attached should though, as mentioned before, be standardised. Further, it might be possible to develop individual settings within AMS since more data is commonly collected during each milking. In turn, the need of individual settings may also be greater within the AMS compared to conventional milking due to the higher milking frequency and uneven milking intervals.

There is a potential in applying individual settings within AMS to improve production efficiency and also further increase udder health due to reduced risk of milking on empty teats. In turn, these two factors are associated with the topic of economic viability within AMS. Profitability in AMS can be increased by increasing milk harvested per milking unit (Jargo et al., 2006). Clean udders in between milking sessions and breeding programs designed to breed cows with a milk ejection reflex that do require minimum amount of stimuli, result in exclusion of pre-stimulation which saves time and increases economic viability. This is common in countries having production systems with once-daily milking where short milking time is particularly important, e.g. Australia and New Zealand (Jargo et al., 2006; Davis et al., 2008). Considering development of individual settings within the AMS and improved attachment of teatcups, this technique allows for short pre-stimulation times (Weiss & Bruckmaier, 2005). In turn, this may decrease the total time spent for individual cows within the AMS. A short pre-stimulation phase when having a high udder filling has the same potential as a long pre-stimulation to generate an increased milk flow rate and quick milking. Thereby, total milking time can be expected to decrease when adding a short pre-stimulation in the milking routine. Individual settings regarding pre-stimulation time is thus interesting in many different production systems and will promote good udder health and increase economic viability throughout the world.

There is a risk of central inhibition due to emotional stress in the cow when switching from suckling to machine milking, which is even greater in systems of restricted suckling when the calf is being tethered (Tančin et al., 2001). The effect of the latency period associated with calf tethering has not been sufficiently investigated. A risk of central inhibition is also likely the case when having the calf tethered next to the cow during the whole milking session. This system has benefits such as reduced risk of lesions when separating the calf from the udder (Combellas et al., 2003), but may still not be optimal. This said, it should be remembered that restricted suckling is used to

feed both humans and the calf, which should be kept in mind when comparing the calf as a pre-stimulator to manual or mechanical stimulation. In turn, it has been found that restricted suckling is associated with a lower frequency of mastitis and sub-clinical mastitis, improved lactation persistency and increased calf growth rate (Krohn, 2001) that may partially compensate for the risk of inhibition of the milk ejection.

Stimulation during the entire milking process is important since the elevated amount of oxytocin results in constant milk ejection during the whole milking session and a high milk yield (Bruckmaier et al., 1994b). Since milk flow affects the vacuum within the liner, it also affects the d-phase. As a consequence, there is less massage of the teats during periods of high milk flow that may potentially influence the concentration of oxytocin. Therefore, it is important to use milking equipment that does not result in a significant reduction in the length of the massage phase during high milk flow in order to maximise milk yield.

Increasing the rate of massage during the final part of milking in modern systems and its effect on milk yield and composition is unknown. In the study by Fjäderhane (1992), an increase in milk yield on udder level could be observed in relation to post-stimulation. A total increase in fat yield within milk from the treated teat could also be observed. This observation is though most likely a result of the high fat yield within the last ten millilitres of milk collected. As there is a common endocrine environment for all quarters within the udder, the increased milk yield is likely a result of more complete udder emptying due to a higher concentration of oxytocin associated with the increased massage. In turn, the increase in fat yield within the last milk collected is also a likely outcome of a more complete udder emptying since there is an exponential increase in fat yield to milk volume (Johansson et al., 1952).

At a more efficient udder emptying, there is less accumulation of serotonin and FIL within the alveoli. Since both serotonin and FIL do decrease milk protein production and disrupt tight junctions, removal of these results in an enhanced milk production. Similar results and reasons explain the increased milk yield that result from increased milking frequency (Österman & Bertilsson, 2003). In turn, removal of FIL and serotonin reduces cell apoptosis within the udder. Thereby, removal of serotonin and FIL may result in more cells being able to bring out de novo-synthesis, representing 40-50% of total milk fat (Sjaastad et al., 2010), and thereby further increase fat yield.

Despite having an increased milk and fat yield as a possible outcome of a more efficient udder emptying, it may also be a result of other unknown mechanisms within the udder. It has been found that there is no difference in amount of residual milk obtained in between hand-milked and machine-milked quarters (Svennersten et al., 1990). Adding these findings to the increase in milk and fat yield found by Fjäderhane (1992), post-stimulation cannot exclusively be explained as a factor that affects the udder emptying. Moreover, it has been found that there is an increase in short-chain fatty acids when performing hand milking of one udder quarter during the entire milking process (Svennersten, 1990). The author explained that this was due to more efficient stimulation of the afferent nerve, causing a release of neuropeptides which in turn increased secretory cell activity and progress through fat synthesising pathways (Svennersten, 1990; Svennersten & Barrefors, 1991). This reasoning may explain the increase in fat yield following post-stimulation as well. In turn, post-stimulation may

also affect the expression of hormone receptors within the udder or have an impact on other factors that may influence milk and fat yield.

Whether post-stimulation results in a more efficient udder emptying or affects unknown mechanisms within the udder remains unknown. What is known is that an effect on milk and fat yield has been observed in connection to post-stimulation, which highlights the need of more research within the area. It is my personal belief that post-stimulation plays a role in stimulating further milk production as the calf performs it during nursing. This statement can further be supported by research where suckling by a calf three times per day combined with a milking frequency of three times per day result in an increased milk yield compared to milking frequency of six times per day (Bar-Peled et al., 1998). The only difference between the two groups is the suckling and teat stimulation. Increased milk yield may therefore be a result of higher concentration of oxytocin and a more complete udder emptying and/or the so far unexplained mystery behind the post-stimulation performed by the calf.

Conclusion

Pre-stimulation is important to induce milk ejection, prevent bimodal milk flow curves and reduce the risk of milking on empty teats. In order to increase milking efficiency, udder health and economic viability, the technique of applying individual settings regarding pre-stimulation time is in need of further development. Pre-stimulation should induce a maintained alveolar contraction that is followed by a milking session where the liner of the milking unit carries out a continuous massage of the teats regardless of the milk flow. This is important in order to maintain a high concentration of oxytocin throughout milking. Development of pulsator settings with a more frequent pressure change during the final minute of milking resembling the post-stimulation massage carried out by the calf could potentially increase milk and fat yield and thereby improve dairy production. Hence, all three suckling activities that the calf displays during nursing are important to consider in machine milking.

References

- Algers, B., Jensen, P. 1985. Communication during suckling in the domestic pig. Effects of continuous noise. *Applied Animal Behaviour Science* 14, 49-61.
- Algers, B., Jensen, P. 1989. Vocal and tactile communication during suckling in pigs. Thesis. Report no 25. Swedish University of Agricultural Sciences, Skara, Sweden.
- Allen, D.B., de Peters, E.J., Laben, R.C. 1986. Three times a day milking: Effects on milk production, reproductive efficiency, and udder health. *Journal of Dairy Science* 69, 1441-1446.
- Ambord, S., Bruckmaier, R.M. 2010. Milk flow-dependent vacuum loss in high-line milking systems: Effects on milking characteristics and teat tissue condition. *Journal of Dairy Science* 93, 3588-3594.
- Bar-Peled, U., Aharoni, Y., Robnson, B., Bruckental, I., Lehrer, R., Maltz, E., Knight, C., Kali, J., Folman, Y., Voet, H., Gacitua, H., Tagari, H. 1998. The effect of enhanced milk yield of dairy cows by frequent milking or suckling on intake and digestibility of the diet. *Journal of Dairy Science* 81, 1420-1427.
- Bar-Peled, U., Maltz, E., Bruckental, I., Folman, Y., Kali, Y., Gacitua, H., Lehrer, A.R., Knight, C.H., Robnson, B., Voet, H., Tagari, H. 1995. Relationship between frequent

- milking or suckling in early lactation and milk production of high producing dairy cows. *Journal of Dairy Science* 78, 2726-2736.
- Bruckmaier, R.M. 2003. Chronic oxytocin treatment causes reduced milk ejection in dairy cows. *Journal of Dairy Research* 70, 123-126.
- Bruckmaier, R.M., Blum, J.W. 1992. B-mode ultrasonography of mammary glands of cows, goats, and sheep during alpha- and beta-adrenergic agonist and oxytocin administration. *Journal of Dairy Research* 59, 151-159.
- Bruckmaier, R.M., Blum, J.W. 1996. Simultaneous recording of oxytocin release, milk ejection and milk flow during milking of dairy cows with and without prestimulation. *Journal of Dairy Research* 63, 201-208.
- Bruckmaier, R.M., Blum, J.W. 1998. Oxytocin release and milk removal in ruminants. *Journal of Dairy Science* 81, 939-949.
- Bruckmaier, R.M., Hilger, M. 2001. Milk ejection in dairy cows at different degrees of udder filling. *Journal of Dairy Research* 68, 369-376.
- Bruckmaier, R.M., Macuhova, J., Meyer, H.H.D. 2001. Specific aspects of milk ejection in robotic milking: a review. *Livestock Production Science* 72, 169-176.
- Bruckmaier, R.M., Rothenanger, E., Blum, J.W. 1994a. Measurement of mammary gland cistern size and determination of the cisternal milk fraction. *Milchwissenschaft* 49, 543-546.
- Bruckmaier, R.M., Schams, D., Blum, J.W. 1993. Milk removal in familiar and unfamiliar surroundings: concentration of oxytocin, prolactin, cortisol and β -endorphin. *Journal of Dairy Research* 60, 449-456.
- Bruckmaier, R.M., Schams, D., Blum, J.W. 1994b. Continuous elevated concentrations of oxytocin during milking are necessary for complete milk removal in dairy cows. *Journal of Dairy Research* 61, 323-334.
- Bruckmaier, R.M., Wellnitz, O., Blum, J.W. 1997. Inhibition of milk ejection in cows by oxytocin blockade, α -adrenergic receptor stimulation and in unfamiliar surroundings. *Journal of Dairy Research* 64, 315-325.
- Bruckmaier, R.M., Wellnitz, O. 2008. Induction of milk ejection and milk removal in different production systems. *Journal of Animal Science* 86, 15-20.
- Collier, R.J., Hernandez, L.L., Horseman, N.D. 2012. Serotonin as a homeostatic regulator of lactation. *Domestic Animal Endocrinology* 43, 161-170.
- Combellas, J., Tesorero, M., Gabaldón, L. 2003. Effect of calf stimulation during milking on milk yield and fat content of *bos indicus* \times *bos taurus* cows. *Livestock Production Science* 79, 227-232.
- Davis, K.L., Fulkerson, W.J., Garcia, S.C., Dickeson, D., Barchia, I.M. 2008. Premilking teat preparation for Australian pasture-based cows milked by an automatic milking system. *Journal of Dairy Science* 91, 2604-2609.
- Dzidic, A., Weiss, D., Bruckmaier, R.M. 2004. Oxytocin release, milk ejection and milking characteristics in a single stall automatic milking system. *Livestock Production Science* 86, 61-68.
- Ely, F., Peterson, W.E. 1941. Factors involved in the ejection of milk. *Journal of Dairy Science* 24, 211-223
- EEC. 1989. European Union Council Directive. Commission Directive 89/362/EEC of 26 May 1989 on general conditions of hygiene in milk production holdings. April 2013. http://faolex.fao.org/cgi-bin/faolex.exe?rec_id=013720&database=FAOLEX&search_type=link&table=result&lang=eng&format_name=@ERALL.

- Fjäderhane, A. 1992. Effekter av för- och efterstimulering på mjölmängd och mjölksammansättning. Swedish University of Agricultural Sciences, department of animal nutrition and management, degree of master of science in agriculture. Degree thesis no. 48.
- Fröberg, S., Aspegren-Güldorff, A., Olsson, I., Marin, B., Berg, C., Hernández, C., Galina, C.S., Lidfors, L., Svennersten-Sjaunja, K. 2007. Effect of restricted suckling in milk yield, milk composition, and udder health in cows and behaviour and weight gain in calves, in dual-purpose cattle in the tropics. *Tropical Animal Health and Production* 39, 71-81.
- Gorewit, R.C., Aromando, M.C. 1985. Mechanism involved in the adrenaline induced blockade of milk ejection in dairy cattle. *Proceedings of the Society for Experimental Biology and Medicine* 180, 340-347.
- Gorewit, R.C., Svennersten, K., Butler, W.R., Uvnäs-Moberg, K. 1992. Endocrine responses in cows milked by hand and machine. *Journal of Dairy Science* 75, 443-448.
- Haley, D.B., Rushen, J., Duncan, I.J.H., Widowski, T.M., de Passillé, A.M. 1998. Butting by calves, *bos taurus*, and rate of milk flow. *Animal Behaviour* 56, 1545-1551.
- Henderson, A.J., Peaker, M. 1984. Feedback control of milk secretion in the goat by a chemical in milk. *Journal of Physiology* 351, 39-45.
- Hernandez, L.L., Stiening, C.M., Wheelock, J.B., Baumgard, L.H., Parkhurst, A.M., Collier, R.J. 2008. Evaluation of serotonin as a feedback inhibitor of lactation in the bovine. *Journal of Dairy Science* 91, 1834-1844.
- Jargo, J.G., Davis, K.L., Copeman, P.J., Woolford, M.M. 2006. The effect of pre-milking teat-brushing on milk processing time in an automated milking system. *Journal of Dairy Research* 73, 187-192.
- Johansson, I., Korkman, N., Nelson, N.J. 1952. Studies on udder evacuation in dairy cows. *Acta Agriculturae Scandinavica* 2, 43-81.
- Johansson, B., Uvnäs-Moberg, K., Knight, C., Svennersten-Sjaunja, K. 1999. Effect of feeding before, during and after milking on milk production and the hormones oxytocin, prolactin, gastrin and somatostatin. *Journal of Dairy Research* 66, 151-163.
- Kaskous, S., Bruckmaier, R.M. 2011. Best combination of pre-stimulation and latency period duration before cluster attachment for the efficient oxytocin release and milk ejection in cows with low to high udder-filling levels. *Journal of Dairy Research* 78, 97-104.
- Knight, C.H. 2001a. Overview of prolactin's role in farm animal lactation. *Livestock Production Science* 70, 87-93.
- Knight, C.H. 2001b. Lactation and gestation in dairy cows: flexibility avoids nutritional extremes. *Proceedings of the Nutritional Society* 60, 527-537.
- Knight, C.H., Hirst, D., Dewhurst, R.J. 1994. Milk accumulation and distribution in the bovine udder during the interval between milkings. *Journal of Dairy Research* 61, 167-177.
- Krohn, C.C. 2001. Effect of different suckling systems on milk production, udder health, reproduction, calf growth and some behavioural aspects in high producing dairy cows – a review. *Applied Animal Behaviour Science* 72, 271-280.
- Lidfors, L.M., Jensen, P., Algers, B. 1994. Suckling in free-ranging beef cattle – temporal patterning of suckling bouts and effects of age and sex. *Ethology* 98, 321-332.
- Linzell, J.L. 1955. Some observations on the contractile tissue of the mammary gland. *Journal of Physiology* 130, 257-267.
- Lupoli, B., Johansson, B., Uvnäs-Moberg, K., Svennersten-Sjaunja, K. 2001. Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin,

- somatostatin and insulin in dairy cows and their calves. *Journal of Dairy Research* 68, 175-187.
- Mačuhová, J., Tančin, V., Bruckmaier, R.M. 2003. Oxytocin release, milk ejection and milk removal in a multi-box automatic milking system. *Livestock Production Science* 81, 139-147.
- Marshall, A.M., Pai, V.P., Sartor, M.A., Horseman, N.D. 2009. *In vitro* multipotent differentiation and barrier function of a human mammary epithelium. *Cell Tissue Research* 335, 383-395.
- Matsuda, M., Imaoka, T., Vomachka, A.J., Gudelsky, G.A., Hou, Z., Mistry, M., Bailey, J.P., Nieport, K.M., Walther, D.J., Bader, M., Horseman, N.D. 2004. Serotonin regulates mammary gland development via an autocrine-paracrine loop. *Developmental Cell* 6, 193-203.
- Mayer, H., Bruckmaier, R.M., Schams, D. 1991. Lactational changes in oxytocin release, intramammary pressure and milking characteristics in dairy cows. *Journal of Dairy Research* 58, 159-169.
- Mayer, H.K., Schams, D., Worstorff, H., Karg, H. 1986. Oxytocin release during prestimulation and milking and its significance for milk removal in dairy cows. *Vlaams diergegeeskundig tijdschrift* 4, 286-296.
- Mayntz, M., Costa, R. 1998. Effect of pharmacologically induced changes in milk ejection on suckling in *bos taurus*. *Physiology & Behaviour* 65 (vol 1), 151-156.
- Mein, G.A. 1992. Action of the cluster during milking. In: *Machine milking and lactation* (eds. A.J. Bramley, F.H. Dodd, G.A. Mein, J.A. Bramley), 97-140. Insight Books, Burlington.
- Mengistu, U. 2004. Performance of the Ethiopian Somali goat during different water regimes. Doctoral thesis. Dept. of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences. *Acta Universitatis agriculturae Sueciae*, vol 2007:53
- Nguyen, D-A.D., Neville, M.C. 1998. Tight junction regulation in the mammary gland. *Journal of Mammary Gland Biology and Neoplasia* 3, 233-246.
- Orihuela, A. 1990. Effect of calf stimulus on the milk yield in zebu-type cattle. *Applied Animal Behaviour Science* 26, 187-190.
- Peaker, M., Wilde, C.J. 1996. Feedback control of milk secretion from milk. *Journal of Mammary Gland Biology and Neoplasia* 1, 307-315.
- Peeters, G., Buyssacher, E., Vandeveld, M. 1973. Milk ejection in primiparous heifers in the presence of their calves. *Zentralblatt für Veterinärmedizin A* 20, 503-536.
- Cited through: Svennersten-Sjaunja, K. 2004. The science behind milk ejection. *NMC Animal Meeting Proceedings*, 215-228.
- Pfeilsticker, H.U., Bruckmaier, R.M., Blum, J.W. 1996. Cisternal milk in the dairy cow during lactation and after preceding teat stimulation. *Journal of Dairy Research* 63, 509-515.
- Phillips, D.S.M. 1984. Studies on pre-milking preparation. 4. Estimation of the pre-milking stimulus requirement of cows. *New-Zealand Journal of Agricultural Sciences* 27, 495-499.
- Pollock, W.E., Hurnik, J.F. 1978. Effects of calf calls on rate of milk release of dairy cows. *Journal of Dairy Science* 61, 1624.
- PubChemCompound. March 2013. Serotonin – compound summary.
http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5202&loc=ec_rcs.
- Rasmussen, M.D. 1993. Influence of switch level of automatic cluster removers on milking performance and udder health. *Journal of Dairy Research* 60, 287-297.

- Rasmussen, M.D., Frimer, E.S., Horvath, Z., Jensen, N.E. 1990. Comparison of a standardised and variable milking routine. *Journal of Dairy Science* 73, 3472-3480.
- Rasmussen, M.D., Mayntz, M. 1998. Pressure in the teat cistern and the mouth of the calf during suckling. *Journal of Dairy Research* 65, 685-692.
- Sagi, R., Gorewit, R.C., Merrill, W.G., Wilson, D.B. 1980. Premilking stimulation effects on milking performance and oxytocin and prolactin release in cows. *Journal of Dairy Science* 63, 800-806.
- Samuelsson, B., Wahlberg, E., Svennersten, K. 1993. The effect of feeding during milking on milk production and milk flow. *Swedish Journal of Agricultural Research* 23, 101-106.
- Sjaastad, Ø.V., Sand, O., Hove, K. 2010. Lactation. In: *Physiology of Domestic Animals*, 2nd edition, 735-760. Scandinavian Veterinary Press, Oslo.
- Stelwagen, K., Knight, C.H. 1997. Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation. *Journal of Dairy Research* 64, 487-494.
- Stull, M.A., Pai, V., Vomachka, A.J., Marshall, A.M., Jacob, G.A., Horseman, N.D. 2007. Mammary gland homeostasis employs serotonergic regulation of epithelial tight junctions. *Proceedings of the National Academy of Sciences* 104, 16708-16713.
- Svennersten, K. (1990). Central and local mechanisms involved in the control of milk production and milk let down. Doctorial thesis. Dept. of Animal Nutrition and Management, Swedish University of Agricultural Sciences. *Acta Universitatis agriculturae Sueciae*, vol 1990:199.
- Svennersten, K., Barrefors, P. 1991. Effect of local stimulation on fatty acid composition in bovine milk. *Milchwissenschaft* 46, 507-509.
- Svennersten, K., Claesson, C.O., Nelson, L. 1990. Effect of local stimulation of one quarter on milk production and milk components. *Journal of Dairy Science* 73, 970-974.
- Svennersten-Sjaunja, K. 1995. Efficient milking. Alfa Laval Agri AB, Tumba, Sweden.
- Svennersten-Sjaunja, K. 2004. The science behind milk ejection. NMC Animal Meeting Proceedings, 215-228.
- Svennersten-Sjaunja, K., Olsson, K. 2005. Endocrinology of milk production. *Domestic Animal Endocrinology* 29, 241-258.
- Tančin, V., Harecek, L., Brouček, J., Mihina, S. 1995. Effect of suckling during during early lactation and changeover to machine milking on plasma oxytocin and cortisol levels and milking characteristics in Holstein cows. *Journal of Dairy Research* 62, 249-256.
- Tančin, V., Kraetzel, W-D., Schams, D., Bruckmaier, R.M. 2001. The effect of conditioning to suckling, milking and the calf presence on the release of oxytocin in dairy cows. *Applied Animal Behaviour Science* 72, 235-246.
- Uvnäs-Moberg, K., Stock, S., Eriksson, M., Lindén, A., Einarsson, S., Kunavongkrit, A. 1985. Plasma level oxytocin increase in response to suckling and feeding in dogs and sows. *Acta Physiologica Scandinavica* 124, 391-389.
- Weiss, D., Bruckmaier, R.M. 2005. Optimization of individual prestimulation in dairy cows. *Journal of Dairy Science* 88, 137-147.
- Weiss, D., Dzidic, A., Bruckmaier, R.M. 2003. Effect of stimulation intensity on oxytocin release before, during and after machine milking. *Journal of Dairy Research* 70, 349-354.
- Weiss, D., Weinfurtner, M., Bruckmaier, R.M. 2004. Teat anatomy and its relationship with quarter and udder milk flow characteristics in dairy cows. *Journal of Dairy Science* 87, 3280-3289.
- Wellnitz, O., Bruckmaier, R.M., Blum, J.W. 1999. Milk ejection and milk removal of single quarters in high yielding dairy cows. *Milchwissenschaft* 54, 309-306.

- Wilde, C.J., Addey, C.V.P., Boddy, L.M., Peaker, M. 1995. Autocrine regulation of milk secretion by a protein in milk. *Biochemical Journal* 305, 51-58.
- Wilde, C.J., Addey, C.V., Knight, C.H. 1989. Regulation of intracellular casein degradation by secreted milk proteins. *Biochemica et Biophysica Acta* 992, 315-319.
- Wilde, C.J., Henderson, A.J., Knight, C.H., Blatchford, D.R., Faulkner, A., Vernon, R.G. 1987. Effects of long-term thrice-daily milking on mammary enzyme activity, cell population and milk yield in the goat. *Journal of Animal Science* 64, 533-539.
- Österman, S., Bertilsson, J. 2003. Extended calving interval in combination with milking two or three times per day: effects on milk production and milk composition. *Livestock Production Science* 82, 139-49.

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