SLU

UPPSALA UNIVERSITY

LACTATION RESEARCH IN MAMMALS AND HUMANS:

THE MAMMARY GLAND IN HEALTH AND DISEASE

WITH PARTICULAR FOCUS ON
MILK EJECTION AND
EMPTYING OF THE MAMMARY GLAND

PROCEEDINGS FROM A SYMPOSIUM IN UPPSALA, SWEDEN DECEMBER 4-5, 2012

Sigrid Agenäs (editor)

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Foreword

CRU is a multidisciplinary network of almost 100 scientists with different academic backgrounds at SLU, Swedish University of Agricultural Sciences, and UU, Uppsala University. During two days in 2008, Nov 13-14, CRU hosted a symposium on "Lactation research in mammals and humans: comparative aspects with focus on milk composition and mastitis" in Uppsala. The meeting between scientists and research students in human and animal lactation and clinicians from both human and animal medicine proved very successful and it was agreed to make this a biannual tradition.

The third biannual lactation symposium organized by the CRU will take place in Uppsala, December 4-5, 2012. The theme for this symposium is "**Lactation research in mammals and humans: the mammary gland in health and disease** with particular focus on milk ejection and emptying of the mammary gland".

Lactation is a fundamental part of reproduction. The composition of milk is important for young both babies and animals, and varies within the lactation period. Optimal nutrition of premature babies is dependent on knowledge of milk composition, and the relationship between milk composition and health later in life is a developing research field. Milk composition in early life of calves is important also for cattle production, and milk composition in dairy cows has a great economical significance for the dairy industry. One way to increase the knowledge about factors influencing milk composition, from synthesis in the mammary gland to handling of milk at the dairy or hospital, is to perform comparative research between human and bovine lactation.

Milk ejection and subsequent removal of milk from the glands is crucial for maintaining the established lactation and these matters are therefore always important to discuss. In human lactation problems with milk ejection and milk removal for example occur in situations that are stressful for the mother or when she is in pain. In management of dairy cows new technology currently raises new questions about milking management.

On behalf of CRU and the organizing committee: Sigrid Agenäs, Gunilla Hallberg, Elisabeth Kylberg, Karin Persson Waller and Bodil Ström Holst wishes you welcome to take part of all aspects of the symposium - oral presentations, panel discussions, posters and informal discussions.

Uppsala, December 2012

Sigrid Agenäs (editor)

CRU SYMPOSIUM ON

LACTATION RESEARCH IN MAMMALS AND HUMANS:
THE MAMMARY GLAND IN HEALTH AND DISEASE

WITH PARTICULAR FOCUS ON MILK EJECTION AND EMPTYING OF THE MAMMARY GLAND

Uppsala, Ultuna, Main building, Lecture room L

4 December

09.00	Registration, coffee	
09.30	Welcome, information	*Sigrid Agenäs*
	SESSION 1 (Moderator Karin Persson Waller)	
09.45	Mammary gland anatomy and endocrine regulation of milk ejection in different species	*Rupert Bruckmaier*
10.35	Short break	
10.45	Milk ejection: Comparative studies with specific reference to women and sows	*Peter Hartmann*
11.35	Influence of residual milk on milk yield and composition in dairy cows	*Sabine Ferneborg*
11.50	Can milk composition and individual cheese yield increase by suckling?	*Madeleine Högberg*
12.15	LUNCH	
	SESSION 2 (Moderator Elisabeth Kylberg)	
13.30	Initiation of breastfeeding after Caesarean section	*Eva Nissen*
14.30	Coffee	
15.00	The importance of mammary secretions in evolution and consequences for the lactating dairy cow.	*Rupert Bruckmaier*
15.30	ISO standards for milking dairy cows at udder quarter level	*Charlotte Hallén Sandgren*
15.50	Panel discussion	
16.15	Conclusions from session 1 and 2	*Sigrid Agenäs*
16.30	End of programme day 1	
17.00	DINNER	

5 December

	SESSION 3 (Moderator Sigrid Agenäs)	
09.30	Milk removal and the innate immune system	*Kerst Stelwagen*
10.20	Mastitis in beef cows – a threat against calf growth?	*Karin Persson Waller*
10.35	Coffee	
11.00	How effective is enrofloxacin against acute clinical mastitis caused by *Escherichia coli*?	*Ylva Persson*
11.15	Infection patterns of *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* in early lactation dairy cows	*Åsa Lundberg*
11.30	Panel discussion	
12.00	Conclusions and CLOSING	*Sigrid Agenäs*
12.15	LUNCH	

MAMMARY GLAND ANATOMY AND ENDOCRINE REGULATION OF MILK EJECTION IN DIFFERENT SPECIES

Rupert M. Bruckmaier✉

Veterinary Physiology, Vetsuisse Faculty University of Bern, Switzerland

✉*rupert.bruckmaier@vetsuisse.unibe.ch*

Mammary glands of all mammalian species consist of secretory tissue, and a duct and cisternal cavity system. The secretory tissue is based on alveolar structures containing secretory epithelial cells and myoepithelial cells which contract in response to oxytocin. The alveoli are connected via small milk ducts with the larger milk ducts, cisternal cavities, teats and the gland orifice. Between the events of milk removal by suckling or milking, milk accumulates in the gland and is stored in the various compartments. While the milk stored in the alveoli and small milk ducts is fixed by adhesive forces (alveolar milk), the milk in the larger ducts and cavities (cisternal milk) is only prevented from draining off the gland by the teat canal smooth muscles.

Availability of milk before milk ejection

The cisternal milk is immediately available by sucking or milking vacuum (negative pressure) while the alveolar milk cannot be removed before it is actively shifted into the cisternal cavities by a positive pressure on the alveoli through myoepithelial contraction in response oxytocin. As the size of the cisternal cavities and hence the cisternal milk fraction differs tremendously between species, the availability of milk prior to milk ejection differs accordingly. In many species such as mouse, pig and human, there are only small sinuses in the larger milk ducts and almost no cisternal milk is present. Small cisterns with a cisternal fraction of < 10 % of total milk are found in horses and water buffaloes, larger cisterns containing up to 30 % of the stored milk are present in dairy cows, and high amounts of cisternal milk are available in sheep (50 %) and goats (up to 80 %). However, also in species with large cisterns the presence of a certain hydrostatic pressure within the alveoli is required that a portion of milk is pressed into the cavities by the alveolar pressure through milk secretion (not milk ejection). Shortly after milk removal by suckling or milking, it takes a few hours until the alveoli are refilled, and milk starts to accumulate in the cisternal cavities. The availability of cisternal milk determines if milk is available right at the start of suckling or milking to bridge the time until alveolar milk ejection, or if milk is only available after tactile stimulation of the mammary gland, oxytocin release, and alveolar milk ejection. Especially in water buffaloes, despite the presence of cisternal milk, an additional teat closure on top of the teat canal prohibits the availability of cisternal milk by milking vacuum application. Thus, milk ejection is required before any milk (including cisternal milk) can be obtained by machine milking. The additional closure of the teat is released in response to an increasing intracisternal pressure caused by milk ejection.

Oxytocin release and milk ejection

Oxytocin is a neuropeptide which is produced in the hypothalamus and stored in the posterior pituitary until released into blood circulation. Its concentration in blood is extremely low as compared to other endocrine factors (1-5 pg/ml (= 10^{-12} g/ml) basal values). Oxytocin has several functions in reproduction such as myometrial contraction during parturition and myoepithelial contraction to cause milk ejection. During suckling oxytocin is released into blood but also within the CNS which is thought to be responsible to induce and maintain a strong bonding between mother and offspring. Oxytocin is released via a neuro-endocrine reflex mainly by tactile stimulation of the mammary gland or the genital tract. Other types of stimulation such as acoustic or

visual stimuli do obviously exist in some species but have never been approved in dairy animals such as cows, sheep and goats. Depending of the type and intensity of stimulation oxytocin increases to levels of 10-100 pg/ml. The effect of oxytocin with respect to milk ejection is not related to the dimension of oxytocin release. It has been demonstrated in dairy cows that surmounting a threshold of approximately 10 pg/ml induces maximum milk ejection. Consequently, there are no special requirements for the intensity of stimulation of the mammary gland to induce a sufficient oxytocin release for maximum milk ejection. Only extremely high oxytocin blood concentrations which can only be achieved by administration of exogenous oxytocin, can induce a more efficient milk ejection to remove also the so-called residual milk, a portion of 5-20 % of the total milk which remains in the udder of cows under physiological conditions.

Time to induce milk ejection

In dairy cows, elevated blood concentrations of oxytocin in the jugular vein can be measured within 30 s after the start of stimulation. However, the lag time from the start of stimulation until alveolar milk is present in the cistern varies widely. It has been demonstrated in dairy cows that with a decreasing degree of alveolar filling the lag time until milk ejection increases. Alveolar milk appears in the cistern after the start of tactile stimulation at about 40-50 s in full udders, and this lag time can reach up to 3 min in udders with a very low degree of filling. This variation is caused by a different degree of myoepithelial contraction which is necessary until milk is pressed out of the alveoli. In dairy practice different degrees of udder filling are caused by the stage of lactation, or by the milking frequency which can even vary in automatic milking systems. A pre-stimulation prior to milking to induce milk ejection before the attachment of the milking machine can be permanent, but can also be split into a short tactile stimulation (minimum 15 s), followed by a latency period until milk ejection occurs (up to 45 s).

Continuous oxytocin release and milk ejection during milk removal

It has been shown in dairy cows that milk ejection continues throughout milking or suckling. Thus milk ejection does not imply a complete shift of alveolar milk into the cistern at the start of milking. The short halflife of oxytocin in blood circulation of only 2-3 min causes an almost immediate cessation of milk ejection when the respective tactile stimulus is terminated. Thus during suckling only the amount of milk is ejected which is required by the offspring. During machine milking the pulsation of the teat cup liner causes a continuous release of oxytocin throughout milking until the udder is empty. However it has to be considered that the gross milk composition changes continuously during milking which is of importance for the somatic cell count and the milk fat content. This fact must be considered for analytical milk sampling.

MILK EJECTION:
COMPARATIVE STUDIES WITH SPECIFIC TO WOMEN AND SOWS

Peter Hartmann✉

School of chemistry and biochemistry, The University of Western Australia, 35 Sterling Highway, Crawley, WA 6009, Australia

✉peter.hartmann@uwa.edu.au

Although Sweden and Australia are half a world apart both countries have very similar high rates of breastfeeding compared to most other developed countries. Both countries had the lowest levels of breastfeeding in 1972 and both now have initiation rates of close to 100% and breastfeeding rates at six months of around 60%. However, the rates of exclusive breastfeeding at 6 months are quite low and are unlikely to improve unless basic knowledge of the physiology of lactation is improved to provide appropriate medical support for breastfeeding mothers. One area that has been investigated perhaps more than others is oxytocin and milk ejection. Even so there are no pathological tests to assess the normal function of milk ejection in women. The importance of milk ejection in dairy animals was recognized from the time of the domestication of animals. Understanding the physiology of milk ejection and indeed milk synthesis and secretion milk was inhibited by the incorrect assumption that milk ejection represented the rapid synthesis of milk. The understanding that milk ejection was the removal of milk stored in the mammary gland was finally established in 1941. It is now known that oxytocin has multiple function that generally relate to promoting calm and connecting responses and opposing the fight, freeze or flight response to stress. Consideration of milk ejection in women and sows is of particular interest because the sow has the highest known control over milk ejection and milk ejection in women has not been extensively studied.

In the sow, oxytocin is released at farrowing. However, it is not released to initiate contractions but rather is released as the result of the contraction of the dilated birth canal after the birth of s piglet. Thus the sow remains calm at this time to allow the piglet to safely move to the udder. The sow suckles about once per hour and once the piglets attach to their favored teats oxytocin is released, intra-mammary pressure increases and milk flows for approximately 15 seconds. Since all glands respond at the same time, stronger piglets cannot challenge weaker piglets during milk flow, thus favoring the survival of large litters.

Many women can sense milk ejection, but 21% of first milk ejections are not sensed, thus the sensing of milk ejection is not a reliable indicator of milk ejection. However, reliable measurement of milk ejection in nursing mothers can be assessed using ultrasound to observe increases in milk duct diameter and by the increase in milk flow rates in mothers expressing their milk. These studies have shown that mothers have distinct patterns of milk ejection that are maintained throughout lactation. Furthermore babies remove most of the milk during the first two milk ejections. Mothers in traditional societies have frequent breastfeeds (more than once per hour) of short duration so it is likely that they usually only have one milk ejection at a breastfeed.

While considering the positive effects of oxytocin it is also important to remember that the very system that keeps people working for the good of others can also promote atrocities such as racism, genocide and war – the “mamma-bear effect”.

My current research is funded from an unrestricted research grant from Medela AG, Switzerland.

INFLUENCE OF RESIDUAL MILK ON MILK YIELD AND COMPOSITION IN DAIRY COWS

Sabine Ferneborg✉, Sigrid Agenäs & Mikko Griinari

Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, 75323 Uppsala

✉*sabine.ferneborg@slu.se*

Introduction

Previous studies have shown that increased milking frequency (IMF) and residual milk removal increases milk yield in dairy cows. IMF increases milk yield due to removal of the feedback inhibitor of lactation (FIL), and it is unknown whether the same mechanism is responsible for the increased milk yield due to residual milk removal. It is also unknown whether the effect of residual milk removal is additive to the effect of IMF.

Aim

To investigate the effect of residual milk removal in combination with two different milking frequencies on milk yield and composition in dairy cows.

Materials and Methods

Four cows were milked 2 or 4 times per day (2x or 4x) with or without residual milk removal (RMR) during four consecutive days in a latin square design. Milk yield was registered and milk samples taken at each milking. The residual milk was removed with the use of oxytocin injections.

Results

Milk yield increased in the treatments 4x, 2xRMR and 4xRMR. The response in milk yield was rapid, with substantial increases already at day 2 of treatment. There were no significant interactions between RMR and IMF and no changes in milk composition in any of the treatments.

Discussion

The effect of residual milk removal appears to be additive to the effect of increased milking frequency and may be governed by another mechanism than FIL. The high milk fat content in the residual milk could be a possible regulatory factor for milk synthesis.

Conclusion

Residual milk removal increased milk yield independent of milking frequency but did not have any effect on milk composition.

CAN MILK COMPOSITION AND INDIVIDUAL CHEESE YIELD INCREASE BY SUCKLING?

Madeleine Högberg✉*, Anders Andrén# , Elke Hartmann* & Kristina Dahlborn*

*Dept of Anatomy, Physiology and Biochemistry, Box 7011, # Dept of Food Science, Box 7051, 750 07 Uppsala, Sweden.

✉*madeleine.hogberg@slu.se*

Introduction

Milk with high fat and casein content is required to achieve maximum cheese yield. Swedish dairy goats generally produce milk with low contents of fat and casein, which results in a low cheese yield.

Aim

The objective of this study was to investigate if milk composition and individual cheese yield can be increased by suckling.

Materials and methods

In study 1, oxytocin levels in plasma during suckling and milking (S) or during milking only (M) was compared. Milk samples from Swedish dairy goats (n=8) were collected continuously from one teat during suckling (S; day 1) and during milking only (M; day 2). Blood samples were collected before, continuously during and after suckling (S) and milking only (M).

In study 2, milk composition and individual cheese yield was measured from12 goats, kept in 4 different MIX systems (milking combined with suckling) during 4 weeks in a cross-over design. In treatment 1 and 2, dams were together with one kid for 16h a day and kids were allowed to suckle before each milking (16hS) or dams were milked before suckling (16hM). In treatment 3 and 4 dams were together with one kid for 9h a day and kids were allowed to suckle before each milking (9hS) or dams were milked before suckling (9hM). Blood samples were analyzed by ELISA and milk samples by a mid-infrared spectroscopy method. Casein content and individual cheese yield was measured by a rennet-coagulation method, where the samples were centrifuged at 1650 x g (15 min 28°C) to remove the whey.

Results

Plasma levels of oxytocin increased during S, but not during M. The milk fat content was higher during S ($P<0.05$) than during M. The individual cheese yield was increased when suckling occurred before milking.

Discussion

This study demonstrates that suckling before or in combination with milking gives a better udder emptying, since the plasma levels of oxytocin were elevated during suckling but not during milking only. In **conclusion**, suckling increase both fat content and cheese yield in Swedish dairy goats.

INITIATION OF BREASTFEEDING AFTER CAESAREAN SECTION

Eva Nissen✉

Department of Women's and Children's Health, Karolinska Institutet

✉*eva.nissen@ki.se*

The frequency of Caesarean section (CS) is increasing worldwide. Studies point at complex differences in socio-demographic background and psychosocial health issues in women giving birth by a CS compared to women giving birth by the vaginal route. Once the CS is performed women feel various amounts of postoperative pain which is difficult to handle in an optimal way in regard to breastfeeding. CS is associated with delayed initiation of breastfeeding and a subsequent shorter breastfeeding period. One critical issue is to implement caring routines which optimize the conditions for mother and child to commence their first breastfeeding. Early skin to skin contact immediately after birth by CS is a measure which provides the most optimal situation for early initiation of breastfeeding. This lecture will present studies on breastfeeding initiation in women with a CS and studies related to early skin-to-skin contact in women with a CS.

THE IMPORTANCE OF MAMMARY SECRETIONS IN EVOLUTION AND CONSEQUENCES FOR THE LACTATING DAIRY COW

Rupert M. Bruckmaier✉

Veterinary Physiology, Vetsuisse Faculty University of Bern, Switzerland

✉*rupert.bruckmaier@vetsuisse.unibe.ch*

Metabolic adaption to milk production

The original role of mammalian lactation in evolution is an intensive care for the newborn to ensure its survival. Suckling provides milk as a complete source of nutrients, energy, vitamins and minerals. Nutrition of the calf has highest priority within the cow's metabolism mainly at the start of lactation when the newborn calf is fully dependent of milk. Even if the nutrient supply for the cow is insufficient, the milk production of the mammary gland is fully maintained, and the cow is mobilizing body tissue to fulfill the nutrient requirements of the mammary gland. This priority is reduced in later stages of lactation concomitantly with a decline of milk yield when the calf is supposed to increasingly feed on roughage to establish ruminal fermentation and to change the entire metabolism from a pseudo-monogastric to a ruminant. If the nutrient resources for the mother are insufficient at this stage, the calf can also survive with a low amount or even without milk.
In the modern dairy cow, breeding for high milk production was most successful during early stages of lactation due to the high metabolic priority ("homeorhesis") of the mammary gland when all available nutrients are directed towards the mammary gland. This is possible because their uptake by the gland functions widely independent of insulin and thus independent of the homeostatic regulation of the cow's organism. As feed intake in early lactation can never cover the needs for energy and nutrients during this period, the cow mobilizes fat and protein in huge amounts, and during hepatic gluconeogenesis a maximum of glucose has to be produced which is needed mainly for the synthesis of lactose in the mammary gland. The required metabolic adaptation is not always fully successful and has tremendously increased the risk of metabolic disturbances and production related diseases during early lactation. In later stages of lactation, the mammary gland underlies increasingly - homeostatic regulation under the influence of insulin, and a deficient uptake of energy and nutrients leads immediately to a reduction of milk production to avoid negative energy balance.

Colostrum formation

During the peripartal phase the first milk plays an additional crucial role: the passive immunization of the newborn via immunoglobulins (IgG) in colostrum to bridge the early weeks of life until the offspring is able to produce own antibodies.
The formation of colostrum follows a defined sequence of events which is regulated by endocrine systems during the period shortly before parturition. The transfer of IgG occurs at an early stage of lactogenesis, and it ceases when synthesis and secretion of lactose, protein and milk fat commences. Breeding for high production levels has mainly focused on the increased secretion of the major milk constituents but not on the independent secretion of IgG. Thus it can be explained that high producing dairy cows have often a lower concentration of IgG in the colostrum than beef cows which have not been breed for high milk production. The volume of colostrum is assumed to be higher in dairy than in beef cows. However, a newborn calf cannot take up more than 2 kg of colostrum, and the additional amount of colostrum including its IgG is abundant and remains unused.

Mother-offspring bonding

Each suckling process contributes to a close bonding between mother and offspring via oxytocin release not only into the blood circulation but also within the CNS.
The effect of the establishment of mother-offspring-bonding during each suckling is also an effect seen in dairy cow. If lactation starts with calf suckling before the cows are moved to machine milking, the release of oxytocin and milk ejection is often reduced during the first milkings after the changeover. Similarly, if a cow got used to a certain way of machine milking routine, disturbed milk ejection occurs in response to changes of the milking environment or milking routine, and most surprisingly disturbed milk ejection also occurs in response to calf suckling. This indicates that there is also an effect such as cow bonding to the milking machine.

Lactation and reproduction

Interactions also occur between lactation and the ovarian cyclic activity postpartum. The survival of the newborn has priority over a potential new pregnancy which is mediated by several endocrine systems. Hormones such as insulin, IGF-1, and thyroid hormones are dramatically changed during the early lactation mainly in high producing animals to mediate the required metabolic adaptations. However, these hormones are also involved in the follicular development and thus re-establishment of a cyclic activity postpartum, and their characteristic changes during metabolic adaptation also seem to have a negative influence on the ovarian activity.

Conclusion

A number of functions related to the survival of the offspring are still highly active in dairy cows bred for high milk production. These need to be considered in animal breeding and animal husbandry.

ARE ISO STANDARDS VALID ALSO WHEN MILKING UDDER QUARTERS SEPARATELY?
A CALL FOR MORE KNOWLEDGE!

Charlotte Hallén Sandgren✉

DeLaval International AB, P.O. Box 39, SE-147 21 Tumba, Sweden

✉*Charlotte.HallenSandgren@delaval.com*

The aim of this presentation is to initiate a discussion around ISO standards in dairy management. The discussion will include the following three main points:

- The example of calf suckling

- Effects of settings on
    - Milking efficiency
    - Teat condition
    - Udder health

- The importance of Milking interval in mastitis management?
    - Prevention
    - Cure

MILK REMOVAL AND THE INNATE IMMUNE SYSTEM

Kerst Stelwagen✉

SciLactis Limited, Waikato Innovation Park, Ruakura Road, Hamilton 3240, New Zealand. *Email:*

✉*kerst.stelwagen@scilactis.co.nz*

Introduction

Milk provides a complete diet for the neonate, which initially is unable to collect, chew or digest solid food. In addition to its nutritional function, milk also provides essential immune protection to the new born. Milk is synthesized by the mammary epithelial cells (MEC) lining an alveolus, the smallest milk producing unit in the mammary gland. Milk collects inside the alveolus and upon removal, be it through suckling or milking, will travel down ducts of increasing size. At the alveolar end milk ducts are of capillary size and for milk to travel down these tiny ducts an external force is required. This force is supplied by myoepithelial cells that contract in response to oxytocin (i.e. the milk ejection reflex). They surround each alveolus and upon contraction "squeeze" the milk out of each alveolus, down the capillary milk ducts. The frequency of milk removal affects not only the total amount of milk produced by the mother, but also the composition of the milk.

Milk removal

In species where milk removal occurs hourly the milk solids content, in particular the milk fat content, tends to be lower and also the energy content of milk content is relatively low (<20 kJ/100 g), as the nutritional requirements of the neonate are met through regular and frequent suckling. In species that suckle their young only once a day or every other day, such as rabbits and tree shrews, milk is more concentrated and in marine mammals that may leave the young unattended for a week or longer milk is very concentrated with the fat content exceeding 50 percent. Such concentrated high energy milk allows the young to survive between feeds.

Modern dairy cows are usually milked twice a day. However, more frequent milking increases milk production by about 20 percent. The optimum appears to be between three and four time per day. In contrast, milking only once daily (ODM), production, relative to twice daily milking, is reduced by approximately 20 percent. The exact causes of these changes in milk production are not known. However, it appears that after approximately 17-18 hours of milk accumulation a number of physiological changes are initiated within the mammary gland (1). Mammary pudic artery blood flow begins to decrease, proteolytic activity in milk increases, a mild inflammatory response is initiated and mammary tight junctions between adjacent MEC become "leaky". Mammary tight junctions are closely linked to the cytoskeleton of the MEC, which in turn is facilitates intracellular movement of cell organelles and secretory vesicles. Less frequent milk removal also compromises the extracellular interaction with the basal surface of the MEC, which in turn compromises intracellular signalling and cytoskeletal function.

Half-udder experiments have shown that these effects are regulated locally within the mammary gland, as adjacent glands, milked or suckled at different frequencies, show different milk production responses. Given that automated milking systems allow individual glands to be milked separately, this information may be particularly important to optimize milk production in individual glands and therefore total production.

Milk removal and mammary innate immunity

Evolutionary the mammary gland appears to have started out as an immune tissue, with the nutritionally function of milk being a much more recent evolutionary phenomenon (2). This may explain why the mammary gland has a very well developed innate immune system. Not only does the mammary gland convey essential immune factors via the milk to the young, it also is key to the protection of the gland itself, as the gland is very susceptible to infection. Not only, does the mammary gland have direct connection (i.e. the teat or nipple opening) with the external environment allowing relatively easy access for pathogens, once inside it provides an ideal environment for pathogens to survive and multiply, as the milk at near body temperature provides an rich culture medium.

From studies where milk removal frequency is reduced, such as with ODM, there is now clear evidence that innate immune factors are up-regulated. Not only factors that have direct antimicrobial activity, such as lactoferrin and defensins, but also factors that may be involved in pathogen recognition and immune signalling, such as RNase 4 and RNase 5 (also known as angiogenin) and acute phase proteins (3). The up-regulation of host-immune factors is likely part of the aforementioned milk inflammatory response associated with ODM. In addition to a biological role, the presence of immune factors in milk also offers scope for developing added-value milk-derived consumer products (4).

Conclusion and summary

The extent of milk removal, be it through suckling or milking, can affect milk composition and milk yield. The effects are regulated locally within the mammary gland and involve changes in cell-cell and cell-extracellular matrix communication.

The mammary gland has a highly developed host-defence system that may not only help to protect the young, but also help to protect the mammary gland against infection. Les frequent milk removal activates the innate immune system in the mammary gland. This may also provide an opportunity to develop value-add dairy products.

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MASTITIS IN BEEF COWS – A THREAT AGAINST CALF GROWTH?

Karin Persson Waller✉[1,2], Ylva Persson[1,3] and Lena Stengärde[4]

[1] Department of animal health and antimicrobial strategies, National Veterinary Institute (SVA), Uppsala; [2] Department of clinical sciences, Swedish University of Agricultural Sciences, Uppsala; [3] Swedish Dairy Association, Stockholm
[4] Swedish Animal Health Service, Kalmar

✉*karin.persson-waller@sva.se*

Introduction

In beef cows, milk production is the most important factor affecting calf growth. Thus, factors that limit production has negative effects on calf weaning weight. Mastitis is a disease reducing milk production and is often associated with bacterial intra-mammary infection (IMI). Studies from abroad indicate that mastitis/IMI significantly affects calf weaning weight. The knowledge on mastitis in Swedish beef cows is, however, very limited.

Aim

The aim of the study was to investigate presence of mastitis/IMI in a small number of beef herds to evaluate the need for more comprehensive studies regarding risk factors and control measures for mastitis in beef cows.

Material and Methods

In 12 herds, udder conformation, condition, and hygiene was investigated and quarter milk samples were taken from 9-12 beef cows approximately one month after calving and close to weaning. The samples were analyzed for somatic cell counts (SCC) and bacteriology using routine culturing procedures. Weight gain in the calves will also be evaluated.

Results

In total, 125 cows were investigated after calving. Of those, 14% had at least one non-lactating udder quarter. Growth of *Staphylococcus aureus*, coagulase-negative staphylococci, or streptococci was found in 15%, 22% and 4% of the cows, respectively. The proportion of udder quarters with IMI was 13%, and the proportion of udder quarters with SCC ≥200000 cells/ml was 18%. All parameters varied markedly between herds. Milk samples close to weaning, as well as weaning weights in calves, will be collected from September to November 2012.

Discussion

The results indicate that the prevalence of intra-mammary infections and the bacteriological findings in Swedish beef cows is in line with studies from USA and UK. Whether this resulted in a lower weaning weight in calves from infected cows is too early to tell. Other studies have reported a reduction of 5-12.5% in weaning weight, and that infection with *S. aureus* causes the largest reduction.

Conclusions

Preliminary results from early lactation indicate that mastitis/udder infection is relatively common in Swedish beef cows. The study is ongoing and will be finished in the end of 2012.

HOW EFFECTIVE IS ENROFLOXACIN AGAINST ACUTE CLINICAL MASTITIS CAUSED BY *ESCHERICHIA COLI*?

Ylva Persson[✉1,2], Marie Mörk[2], Jörgen Katholm[3], Håkan Landin[2]

[1]Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute, Sweden
[2]Swedish Dairy Association
[3]Knowledge Center for agriculture, Cattle, Denmark

✉*ylva.persson@sva.se*

Introduction

In Sweden, acute clinical mastitis caused by *Escherichia (E.) coli* is often treated with enrofloxacin. Treatment of coliform mastitis has been investigated in many studies, often with contradictive results. However, many authors claim that use of antibiotics is an ineffective way to cure coliform mastitis. No double-blinded trials have been performed to prove the effectiveness of enrofloxacin in the treatment of coliform mastitis.

Aim

To study the effectiveness of enrofloxacin in the treatment of acute clinical mastitis caused by *E. coli* in a clinical placebo trial.

Material and Methods

Dairy cows (n=117) with severe acute clinical mastitis, with suspected *E. coli*, were included in the study. Milk samples were collected from the affected udder quarter for further bacteriology. All cows were treated with a test substance of either enrofloxacin or placebo. Additionally, all cows were treated with meloxicam. A treatment protocol was filled in and sent to SVA. Cows with growth of *E. coli* were followed up at day 4, 22 and 28 with new protocols and milk sampling (d 22 and 28).
Differences between treatment groups were tested using Fisher's exact test for categorical factors and Kruskal-Wallis equality-of-populations rank test for continuous variables.

Results

Fifty-six cows had growth of *E. coli*. Thirty-four (60.7%) were treated with enrofloxacin (T1) and 22 (39.3%) were treated with placebo (T0). Six (17.6%) of the T1-cows and three (13.6%) of the T0-cows died within the first days ($p=0.5$). The incidence of paresis was higher among the cows that died ($p=0.04$). At the follow-up at day 4, day 22 and day 28 the T1-group had lower proportion of cows with clinical signs, with a CMT ≥ 3, with visible milk changes and with a positive *E.coli*-growth but the differences were not statistically significant ($p>0.1$). There was a statistically significant difference in milk production between the treatment groups at follow-up at day 22 and day 28.

Discussion and Conclusions

There was no difference in survival between cows treated with enrofloxacin or placebo. Cows treated with enrofloxacin had a higher milk yield at follow up. Long term effects will be analyzed before we can come to any conclusions.

INFECTION PATTERNS OF *STAPHYLOCOCCUS AUREUS*, *STREPTOCOCCUS DYSGALACTIAE* AND *STREPTOCOCCUS UBERIS* IN EARLY LACTATION DAIRY COWS

Åsa Lundberg✉[1,2], Helle Ericsson Unnerstad[1] & Karin Persson Waller[1,2]

[1]Department of animal health and antimicrobial strategies, National Veterinary Institute (SVA), Uppsala, Sweden
[2]Department of clinical sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

✉asa.lundberg@sva.se

Introduction

Mastitis is the most common disease among dairy cows and is often caused by bacterial intramammary infections (IMI). Such IMI are particularly common in early lactation and it is of special importance to prevent these infections since they can have a long-lasting negative influence on milk yield and udder health.

Aim

To investigate when IMI with *Staphylococcus aureus* (Sa), *Streptococcus dysgalactiae* (Srd) and *Streptococcus uberis* (Sru) occur relative to calving, and if the infection patterns vary over the year or due to parity.

Material and methods

During one year, quarter milk samples were collected from 740 cows in 13 problem herds before first milking after calving (day0) and again four days later (day4). Samples were sent to the laboratory for culturing and identification of Sa, Srd and Sru.

Results

Sa was found at both samplings in 85 cows (11.4%). In addition, 50 cows (6.8%) were positive only at day0 and 63 cows (8.5%) were positive only at the second sampling. Srd was identified at both samplings in 55 cows (7.4%), while 52 cows (7%) were positive only at day0 and 17 cows (2.3%) were positive only at day4. Eighteen cows (2.4%) were Sru-positive at both samplings. In addition, 41 cows (5.5%) were positive only at day0 and 17 cows (2.3%) were positive only at the second sampling. There were no differences between parities except for the increased likelihood of culturing Sru from older cows compared to first parity cows at day0. Some seasonal differences were noted.

Discussion

It is likely that cows positive at both samplings had a true IMI. It is more difficult, however, to interpret the results when cows were positive only at one sampling. This could have been due to a healed or emerging IMI, or to sources of error such as contamination at sampling and variable shedding patterns.

Conclusions

Preliminary data show that IMI, particularly infections with Sa and Srd, are common already at calving in problem herds in all parity cows. Streptococci were more common at day0 than day4. In addition, some seasonal variation was found for all pathogens.

MATERNAL TRANSFER OF BMAA VIA MILK TO SUCKLING OFFSPRING: A COMPARISON OF THE L- AND D-ENANTIOMER IN MICE

Marie Andersson[1]✉, Oskar Karlsson[1,2], Ulrika Bergström[1], Eva B Brittebo[2], Ingvar Brandt[1]

Departments of Environmental Toxicology[1] and Pharmaceutical Biosciences[2], Uppsala University, Uppsala, Sweden

✉*marie.andersson@ebc.uu.se*

The cyanobacterial neurotoxin β-methylalanine (BMAA) has been proposed to be involved in the etiology of amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC) in humans, presumably following dietary exposure to BMAA. Subsequent experimental studies in vivo revealed that L-BMAA is a developmental neurotoxicant that induces behavioral disturbances, altered protein expression and degenerative changes in the hippocampus of neonatally exposed adult rodents. Recent field studies in Florida waters and the Baltic Sea further show that BMAA is widely present in aquatic organisms including fish, and there is emerging evidence to suggest that dietary intake is a major route of exposure in humans.

In the present study we compared the excretion of ^{14}C-labeled β-methyl-L-alanine and β-methyl-D-alanine in milk of lactating mice and the subsequent transfer to the developing brain of suckling pups. Using the mouse mammary gland epithelial cell line HC11, we also characterized the kinetics of the uptake of the L and D enatiomers, and the subsequent secretion from HC11 cells loaded with the enatiomers.

Lactating mice were injected i.v. with ^{14}C-labeled L-and D-BMAA at 9 days post partum. Suckling pups were sampled at 1, 3, 8 and 24 h after dose, and the radioactivity in milk collected from the stomach and in brain tissue was measured. The results revealed a rapid excretion of both ^{14}C-labeled enantiomers in milk. Interestingly, L-BMAA was excreted more efficiently than D-BMAA in milk and also showed a higher uptake than D-BMAA in brain tissue at all time-points examined. Mouse mammary gland epithelial cells were shown to accumulate L-BMAA at a 5 times higher rate than D-BMAA following exposure at 37°C. When the cells were exposed at 4°C, the rates of accumulation of both L- and D-BMAA were drastically reduced, suggesting active transport system for both. Enantiomer-specific differences were also observed when the efflux of BMAA was examined in cells preloaded with the labeled enantiomers. The results suggest that the mother-to offspring transfer of the L-enantiomer via milk is more efficient than that of the D-enantiomer, presumably because of the different transport systems they seem to utilize. Given that L-BMAA is a developmental neurotoxicant in rodents the uptake of L-BMAA in the brain of suckling pups suggests that studies on the excretion of BMAA into human breast milk are warranted.

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THE INFLUENCE OF PROLONGED ACTION VITAMIN-MINERAL SUPPLEMENTATION ON THE PRODUCTION OF MILK

Elena Korochkina✉

Department of Clinical Sciences, Swedish University of Agricultural Sciences and Saint Petersburg State Academy of Veterinary Medicine

✉*e.kora@mail.ru*

Introduction

The aim of the present investigation was to determine the effects of intraruminal vitamin-mineral boluses with prolonged action, administered to dry cows according to milk yield in the previous lactation.

Materials and Methods

A randomized clinical trial including 30 dry Holstein cows on a farm in St.Petersburg, Russia, was used to evaluate the effect of prolonged action vitamin-mineral boluses on milk production. Cows were allocated to one of six treatment groups receiving a control diet or slow-release intraruminal boluses containing different vitamin and mineral mixes. Boluses were administered once in the beginning of the dry period (2 months before parturition) All treatments except "Calcium Bolus Extra" which was administered twice: first in the beginning of the dry period and again two days after parturition. Mean values and standard error of 305 d milk yield were calculated (Statistica 6.0, StatSoft). Analysis of variance has not yet been carried out.

Results

Table 1. The effect of prolonged action vitamin-mineral preparations on the milk production.

Group	305d milk yield, kg	
	4th lactation (before treatment), M±m	5th lactation (after treatment), M±m
Group 1 (Cu, Co, Se, Mn, Zn, Vit A, D_3, E)	4943(±704)	5202(±1073)
Group 2 (Cu, Co, SE, Mn, Zn, I, Vit A, D_3, E, Biotin)	7700(±829)	7889(±744)
Group 3 (Cu, Co, Se, I)	7554(±1045)	7667(±1200)
Group 4 (Calcium)	6415(±1150)	6470(±1184)
Group 5 (Cu, Co, Se, Mn, Zn, I, Vit A, D_3, E)	5461(±949)	5207(±533)
Control group (diet)	7621(±1488)	7227(±1227)

Discussion

The preliminary results on the effect of the different vitamin and mineral bolus treatments during the dry period suggests that the vitamin and mineral supplements increased milk yield in the following lactation. 305 d milk yield after bolus treatment was increased by 260 kg in the first group compared with the lactation before bolus treatment; in the second group was up to 189 kg; in the third group was up to 112.2 kg; in the fourth group was up to 55.2 kg. It is likely that these increases were a result of improved vitamin and mineral metabolism of cows.

Also, there is a proposed mechanism of action of vitamin-mineral bolus containing biotin (group 2) suggesting that the increased milk production was a consequence of improved energetic function of the rumen. In its physiologically active form biotin is attached at the active site of four important enzymes, known as carboxylases (Chapman-Smith A, Cronan JE, 1999). Each carboxylase catalyzes an essential metabolic reaction. As a cofactor of enzymes required for fatty acid synthesis, biotin may increase the utilization of glucose to synthesize fats. Biotin has also been found to stimulate glucokinase, an enzyme in the liver, resulting in increased synthesis of glycogen, the storage form of glucose.
The 254 kg decrease in 305 d milk yield between lactations in group 5, that received the Cu, Co, Se, Mn, Zn, I, Vit A, D_3, E treatment was unexpected.

Conclusion

These data are interpreted to indicate that the intraruminal administration of a bolus containing needful vitamins and minerals to milk production cows had a significantly positive response on their milk production. Analysis of variance needs to be performed on the data to confirm the preliminary results.

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