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*Lactation research in mammals and humans:  
Comparative aspects with focus on milk  
composition and mastitis*

Proceedings from a symposium in Uppsala, Sweden  
November 13-14, 2008

Elisabeth Kylberg, Bodil Ström Holst and Kerstin Svennersten Sjaunja (*editors*)

Uppsala, 2008

CRU Report 22

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CENTRE FOR REPRODUCTIVE BIOLOGY IN UPPSALA



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## Foreword

During two days, 13-14 November 2008, the symposium: “Lactation research in mammals and humans: comparative aspects with focus on milk composition and mastitis” is arranged by CRU, Centre of Reproductive Biology in Uppsala. CRU is a multidisciplinary network of almost 100 scientists with different academic backgrounds at SLU, Swedish University of Agricultural Sciences, and UU, Uppsala University.

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.

Mastitis is another important field within lactation research. Mastitis in cows is of great economical and animal welfare importance. Treatment of mastitis is also an important topic, regarding the prudent use of antibiotics to avoid antimicrobial resistance. In human, mastitis leads to both economic and health problems for the society. Better methods to prevent, diagnose and treat mastitis are needed. We hope that the bringing together of researchers working with different aspects of lactation in animals and humans will lead not only to exchange of information, but also to new ideas and future cooperation.

On behalf of CRU and the organizing committee: Kerstin Hedberg Nyqvist, Elisabeth Kylberg, Matts Olovsson, Bodil Ström Holst and Kerstin Svennersten Sjaunja, we wish you welcome to take part of all aspects of the symposium - oral presentations, posters and informal discussions.

Uppsala, November 2008

*Elisabeth Kylberg, Bodil Ström Holst and Kerstin Svennersten Sjaunja (editors)*





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## **Recent findings in farm animal lactation**

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In the last decade the emphasis in European dairy production has moved away from production to focus on product quality, with emphasis on cow and consumer health and welfare. This review will consider how modern technologies such as automated milking, on-line health and fertility monitoring and computerized management systems are helping to improve cow welfare. Intensive lactation cycles impose considerable metabolic loads on dairy cows, so the review will also consider alternative strategies such as extended lactation and extensive production systems. A large database of knowledge and expertise has been built up that has relevance to emerging human lactation problems such as mastitis, and the review will consider how this information might best be used in the future. Observations first made in cattle regarding negative relationships between adiposity and lactation are now being repeated in women, and the underpinning biological mechanisms will be explored. Finally, the potential for individual milk components including bioactive proteins/peptides and certain fatty acids to have positive and/or negative impact on the short- and long-term health of the consumer will be briefly reviewed.

## Recent findings in human lactation

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Human history in Australia is either very old or very young. The Australian Aboriginals date back more than 40,000 years whereas European settlement dates back only 220 years. Unfortunately, the latter has greatly influenced the former in relation to the practice and importance of breastfeeding. Western Australia is about one third the land area of Australia and as a result the health system experiences considerable difficulties in providing health care for pregnant and lactating women over large distances. Currently there is considerable support for breastfeeding mothers because of the benefits that are derived from breastfeeding for both the mother and her baby. Although most mothers (93%) choose to breastfeed their babies, about 40% of these mothers have stopped breastfeeding by 6 months after birth. Thus now virtually all mothers know that breastfeeding is best for their babies, the challenge for the future is to ensure that all mothers have a satisfying breastfeeding experience. In this respect it would seem that there is still much to be learnt from our original inhabitants – the Australian Aboriginals.

The ability to fully nourish their young with milk in any habitat that can support adult life is a common feature of mammals. However, lactation is an ancient reproductive trait that predates the origin of mammals. Although a variety of theories have been proposed to explain the origin of the mammary gland, its functional and morphological origins remain speculative. Most theories conclude that the mammary gland evolved from sweat and sebaceous glands associated with hair follicles. Recently an interesting alternate theory proposed that lactation evolved from the inflammatory response - the highly conserved innate immune response protecting the integuments of early soft skinned animals. It is argued that this response developed into mucus secreting glands that provided protection for both the skin as well as the hatchling and new born and finally to more complex glands providing innate immune protection as well as nourishment for the newborn. This hypothesis is supported by the biochemical evidence that milk contains almost all components of the innate immune system and that two of these components; xanthine oxido-reductase and lysozyme had crucial roles in the development of the nutritional components in milk. Indeed, most of the nutritional components in milk also have either profound or residual innate immune activity.

Nevertheless, there are large variations between mammalian species in the duration of lactation and the composition of the milk they produce. The composition of milk varies

greatly between different species of mammal but is adapted to the specific need of each species young. Most research in human lactation has focuses on breastmilk and its role in promoting optimal growth and development for the baby. In contrast, comparatively little research has been carried out on the anatomy, metabolism and physiology of the lactating human breast because the investigation of human lactation requires the development of non-invasive methods that do not disturb the breastfeeding relationship between the mother and her baby.

The two key events in the establishment of successful lactation are secretory differentiation (lactogenesis I) and secretory activation (lactogenesis II). Secretory activation is the time during pregnancy that the breast develops the capacity to synthesize the unique products in milk such as lactose and  $\alpha$ -lactalbumin, and secretory activation is the initiation of copious milk secretion and occurs after birth in women. Both secretory differentiation and secretory activation appear to be under hormonal control.

Dr Donna Geddes has used ultrasound to image the structure and function of the lactating breast. This non-invasive technique has provided interesting information on the gross anatomy of the lactating breast in relation to the distribution of milk ducts and the relationship between secretory and fatty tissue within the lactating breast. In addition, it was found that milk ejection could be visualized as the sudden expansion of the milk ducts during either a breastfeed or the expression of the breast using a breast pump.

Most women are able to produce more milk than their babies require. Thus as lactation is established mothers need to down regulate their milk synthesis to match their infants appetite. Evidence suggests that milk supply is controlled by the infant's appetite by an autocrine control mechanism. However, much more research is required to enable an understanding of the regulation of milk synthesis, secretion and removal in women to enable the lactating mammary gland to attain the same status in clinical medicine as other major organs of the human body

Research Funding Medela AG Switzerland.

# **Mammary development and remodelling in primiparous (PP) and multiparous (MP) dairy goats during lactation**

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## **Introduction**

Milk production is generally lower, but lactation persistency higher in PP than MP ruminants. This may be related to differences in development and maintenance of mammary gland function, but the underlying mechanisms are not well understood.

## **Aim**

To elucidate whether differences in lactational performance between PP and MP mammary glands are related to the time course of development and maintenance – not only of the mammary epithelial cell (MEC) population, but also of the mammary vasculature which sustains synthetic activity.

## **Material and methods**

Mammary biopsies were obtained from both mammary glands of 3 PP and 12 MP ( $\geq 2$  parity) dairy goats at parturition (d1), days 10, 60, and 180 of lactation. Gene transcription relating to MEC turnover and vascular function was quantified (qRT-PCR), mammary morphology characterised (quantitative histology), and cell turnover determined (TUNEL and Ki-67). Data were analyzed using SAS mixed models.

## **Results**

At parturition, PP compared to MP glands had less alveoli, lower expressions of LALBA, PRL-R, VEGF-R1, RTK, IGFBP3, and TBXAS, but >10-fold higher cell proliferation rates of MEC. At d10 cell proliferation, expression levels of PRL-R, IGF1, LPT, LPT-R, VEGF-R2, ANGPT1, ANGPT2, COX1, PTGIS, LALBA were highest in PP. By mid-late lactation, PP glands had markedly lower rates of apoptosis, more vasculature and fully differentiated MEC than MP.

## **Discussion and conclusions**

MEC formation, angiogenesis and hence lactogenesis appear to be delayed in PP mammary glands. The reason why, is still unresolved. However, mammary development continues further into lactation in PP than MP glands. This combined with improved MEC survival explains higher lactation persistency.



# **The effect of estrogen on lactose in plasma and urine in dairy cows in late lactation**

Agenäs S, Lundström I, Holtenius K

*Department of Animal Nutrition and Management, SLU, Uppsala*

## **Introduction**

Lactose in plasma indicates leakage of lactose across the mammary epithelium. This happens for example in late lactation, possibly caused by the influence of placental hormones.

## **Aim**

The aim of this study was to investigate the effect of exogenous estrogen on mammary tight junctions in cows in late lactation.

## **Material and Methods**

Two studies were performed. One including five non-pregnant cows receiving injections with 17 $\beta$ -estradiol for six days. The effect of exogenous 17 $\beta$ -estradiol on milk yield, milk composition and lactose in plasma and lactose in urine was investigated before, during and after the treatment. The second study included 10 cows and was designed to investigate normal levels of circulating estrogen, lactose in plasma and lactose in urine around day 200 of the pregnancy and around day 300 in lactation where the normal values of circulating estrogen in plasma and lactose in plasma and urine was registered. The normal values were compared with the values after treatment.

## **Results**

Milk yield decreased already the first day after estrogen was injected. Lactose in plasma and urine increased in the end of the injection period. A high correlation between lactose in urine and plasma was found. More than 30% of the total lactose production was lost in urine after estrogen treatment.

## **Discussion & conclusions**

The data shows that exogenous estrogen impairs milk synthesis and mammary tight junction integrity. There was a delay between the decrease in milk yield and opening of tight junctions, indicating that other factors are involved.

# **The Use of Ultrasound Imaging to assess Gastric Volume and Gallbladder Contractibility in Term Breastfed Infants**

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## **Background**

Ultrasound imaging is a non-invasive method of measuring the infant stomach and gallbladder. Little is known about the influence of breastmilk composition on both gastric emptying and gallbladder contraction in term infants.

## **Aim**

To develop and validate ultrasonic measurement of infant stomach volume and to determine the relationship between gallbladder contractibility and fat content of a breastfeed.

## **Materials and Methods**

Sagittal and transverse ultrasound scans (ACUSON, XP10; Toshiba, Aplio 80) of both the infant stomach (n=22) and gallbladder (n=7) were performed before and after a breastfeed. A small milk sample was taken before and after the breastfeed. The cream content was measured by the creatocrit method and the fat content (g/L) calculated as  $5.37 \times \text{crematocrit} + 5.28$ . Milk intake was measured by the test-weigh method.

## **Results**

Milk intake ( $57.0 \pm 19.7\text{g}$ ) correlated well with stomach volume determined by ultrasound ( $r=0.56$ ,  $P=0.007$ ). Reduction in gallbladder volume ( $57 \pm 20\%$ ) was not related to the fat content of the feed ( $4.3 \pm 2.6\text{ g}$ ).

## **Discussion**

Preliminary results indicate ultrasound is a relatively accurate method for the measurement of infant gastric volume and could be used to monitor gastric emptying in relation to breastmilk composition. Gallbladder contraction is generally increased in response to increasing fat content of a meal however this was not evident in this study.

## **Conclusion**

Ultrasound is a reliable non-invasive method of determining gastric volume in breastfed infants. Preliminary results suggest the fat content of a feed may not influence gallbladder contraction although this requires further investigation.

This study was funded by Medela AG.

# **Moderate prematurity promotes catch-up growth in SGA infants up to 40 weeks postmenstrual age**

Funkquist E-L, Tuvemo T, Jonsson B, Serenius F, Nyqvist KH

## **Introduction**

It is assumed that small for gestational age (SGA) infants have a critical period for programming of growth trajectory early in infancy. However, little is known about the benefits and drawbacks of nutrition intervention during this period. Appropriate feeding of SGA infants postnatally needs further highlighting as it may well affect their future health.

## **Aim**

The purpose of this study was to evaluate the growth of infants born SGA related to two different feeding regimens during their hospital stay.

## **Methods**

A retrospective, descriptive and comparative procedure was used to assess the consequences of different nutrition regimens affecting SGA infants at the neonatal units of two Swedish hospitals

## **Material**

The growth of 42 SGA infants (20 moderately premature) was evaluated at two hospitals up to the corrected age of 18 months. At one hospital, infants were fed according to a proactive nutrition regimen stipulating 200 ml milk/kg per day from day two in order to achieve better weight gain.

## **Results**

Infants fed according to the proactive regimen had lower weight loss and regained their birth weight earlier, but did not show better catch-up growth subsequently. Moderately premature SGA infants showed catch-up growth before 40 weeks postmenstrual age. Low gestational age at birth explained less negative standard deviation score for length up to a corrected age of 18 months.

## **Conclusions**

Although infants fed according to a proactive regimen with liberal volumes of milk during the first days had lower weight loss and regained their birth weight earlier, no evidence was found that this would result in a different pattern of growth in later life. It may be assumed that being born SGA and moderately premature is associated with better growth during infancy.

## **Exclusive Breast-Feeding and Infant Vitamin B-12 Status: the MINIMat Trial in rural Bangladesh**

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### **Introduction**

It has been questioned whether infants born to vitamin B12 deficient mothers can maintain adequate vitamin B12 status with the currently recommended duration of exclusive breast feeding (EBF) for 6 months.

### **Aim**

To assess the association between duration of EBF and infant vitamin B12 deficiency in a population where vitamin B12 deficiency is prevalent among pregnant women.

### **Material and Methods**

Based on monthly feeding recalls of the mother, infants were classified as EBF if they had been given breast milk only. Prelacteals were allowed in the definition. Blood was collected from infants at 6 months and plasma was analyzed for vitamin B12 (n=1033). Differences in prevalence of deficiency (<148 pmol/L) between infants with <4 months and 4-6 months EBF were evaluated in a logistic regression model.

### **Results**

The risk of becoming B12 deficient was higher for infants EBF for 4-6 months (33.7 %) than for infants EBF <4 months (27.9 %) (OR= 1.32, P= 0.043). However, the apparently higher risk for B12 deficiency with longer duration of EBF did not remain when adjusting for socioeconomic status (OR=1.25, p=0.096).

### **Discussion**

In the overall sample, we did not observe an association between duration of EBF and infant vitamin B12 status. However, in future analyses we will address the possible impact of maternal B12 status and prenatal micronutrient supplementation on the relation between duration of EBF and infant B12 status.

### **Conclusion**

In this population, infant vitamin B12 deficiency was prevalent but did not appear to be associated with duration of EBF.

# **Mechanisms Implicated in the Reduced $\beta$ -casein Gene Expression in Cadmium Treated Secreting Murine Mammary Epithelial Cells**

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## **Introduction**

We recently demonstrated a negative dose-response relationship between cadmium exposure and  $\beta$ -casein gene expression in mammary glands of lactating mice, indicating that cadmium may disturb milk protein synthesis.

## **Aim**

To examine whether the reduced  $\beta$ -casein gene expression involves a direct effect by cadmium on the mammary epithelium by applying secreting murine mammary epithelial cells (HC11).

Materials and Methods: HC11 cells were treated with non-toxic concentrations of cadmium, 17 $\beta$ -estradiol, ethynylestradiol, tamoxifen, nifedipine or capric acid during 72 h of differentiation and  $\beta$ -casein gene expression was measured by quantitative real-time RT-PCR.

## **Results**

Cadmium treatment reduced  $\beta$ -casein gene expression in a concentration dependent manner in the HC11 cells. No effects on  $\beta$ -casein gene expression were observed by estrogens or the anti-estrogen tamoxifen. Nifedipine and capric acid both reduced  $\beta$ -casein gene expression in a concentration dependent manner. An interaction effect was detected between nifedipine and cadmium on the reduced  $\beta$ -casein levels.

## **Discussion**

The present study demonstrates that cadmium disturbs  $\beta$ -casein gene expression in the lactating mammary tissue directly. Our results suggest that the mechanism for the reduced  $\beta$ -casein gene expression in the cadmium treated HC11 cells involves a decreased availability of calcium, necessary both for  $\beta$ -casein synthesis and for maintenance of the impermeable paracellular barrier of the mammary epithelium.

## **Conclusion**

Our results indicate that cadmium affects calcium levels in secreting mammary cells and by this means affect milk protein synthesis.

## **Mastitis in farm animals**

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Mastitis is an important disease in several farm animals, but especially in dairy cows. Each year, a large number of cows are affected by clinical or subclinical mastitis with negative effects on animal welfare, milk production, milk quality and farmer economy. In addition, antimicrobials are often used in treatment of mastitis. Thus, this disease may be important for the development of antimicrobial resistance. Most cases of mastitis are associated with bacterial infections, but mastitis is a multi-factorial disease with a large number of predisposing factors complicating its control. Such factors can be found in the management, (e.g. milking routines), and environment (e.g. hygiene) of the cow, but factors related to the cow itself (e.g. udder conformation) are also important. Predisposing factors resulting in bacterial contamination of udder and teats, and/or negative impact on the local and general immune systems of the cow are most important. The presentation will give an overview of the incidence, etiology, and pathogenesis of mastitis, and highlight some important areas of ongoing research such as diagnostics of infectious agents and inflammation, disease control related to preventive measures and therapy, and milk quality of relevance for the dairy industry.

## **Mastitis in man**

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Acute mastitis occurs with quite variable frequency. It may be as common as up around 30% of breastfeeding mothers in the West, but quite uncommon e.g. in our study in Pakistan. This condition can be very painful and damaging for well functioning breastfeeding. Presumably the cause is blocking of ducts in the mammary gland giving rise to inflammation, due to the increased pressure in the ducts, followed by tissue damage. Mastitis may vary from a relatively short-lasting, but painful inflammatory process to an intense, longer lasting severe engagement with intense symptoms. If infection occurs in the distended and blocked tissue, an abscess may be formed, which requires antibiotic treatment.

We have in a well controlled study of limited size shown that antiseecretory factor (AF) can significantly protect against the appearance of mastitis.

Subclinical mastitis is an even less well understood condition, which is defined by a ratio of Na/K in milk of  $>1.0$  and is linked to an increased levels of IL-8 in the milk. This condition impairs the child's weight gain and has been associated with asymptomatic inflammation in the mammary tissues, lactation failure, suboptimal growth in early infancy and an increased risk of mother-to-child transfer of HIV. In the bovine species this form of mastitis is linked to decreased milk production.

# **Mastitis among breast feeding women – causes, consequences and management**

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## **Background**

Mastitis is probably the most common acute disease among breast-feeding women. The purpose of this study was to investigate bacteriological findings, clinical symptoms, possible risk factors, complications, treatment and compliance among women with symptoms of lactation mastitis.

## **Methods**

The study included 54 women with symptoms of lactation mastitis. The first visit included clinical investigation, breast milk sampling and, if present, swab sampling of cracked nipples. The women filled out a questionnaire at home daily until they had recovered. A follow-up interview was performed by telephone. The control group consisted of 207 healthy breast milk donors.

## **Results**

Pain was the most frequent symptom among the women with mastitis. In all, 69 % of the women obtained maximum score for pain during the first investigation. *S. aureus* was detected significantly more often in the milk of the women with mastitis than among the healthy controls (48 % vs. 10 %). There were no significant association between clinical symptoms and presence of *S. aureus* in the milk. However, there were a significant association between cracks and nipple sores and presence of *S. aureus* in the milk.

## **Conclusion**

Mastitis is an acute condition which can cause severe pain, fever and malaise throughout several days. It is difficult to differentiate between a non-infectious mastitis from an infectious mastitis by means of clinical symptoms. The presence of cracks and nipple sores increases the likelihood of a bacterial infection. Dicloxacillin is an adequate choice for antimicrobial therapy of a bacterial breast infection.



## **The role of bacteria in lactational mastitis and some considerations of the use of antibiotic treatment**

Kvist LJ, Wilde Larsson B, Hall-Lord ML, Steen A, Schalén C

*International Breastfeeding Journal* 2008 Apr 7; 3:6.

### **Background**

The role of bacterial pathogens in lactational mastitis remains unclear. In the 1980s researchers proposed a division of mastitis into; milk stasis, non-infective mastitis, infective mastitis and breast abscess. The objective of this study was to compare bacterial species in breast milk of women with mastitis and of healthy breast milk donors. Further, to evaluate the relationship of bacterial counts to the symptoms of mastitis and occurrence of breast abscess.

### **Methods**

In this descriptive study, breast milk from 192 women with mastitis (referred to as cases) and 466 breast milk donors (referred to as controls) was examined bacteriologically and compared using analytical statistics.

### **Results**

Five main bacterial species were found in both cases and controls. More women with mastitis had *S. aureus* and GBS in their breast milk than those without symptoms, although 31% of healthy women harboured *S. aureus* and 10% had GBS. There were no significant correlations between bacterial counts and the symptoms of mastitis. There were no differences in bacterial counts between those prescribed and not prescribed antibiotics or those with and without breast abscess. GBS in breast milk was associated with increased health care contacts ( $p = 0.02$ ). Women with  $\geq 10^7$  cfu/L CNS or viridans streptococci in their breast milk had increased odds for damaged nipples ( $p = 0.003$ ).

### **Conclusion**

Many healthy breastfeeding women have potentially pathogenic bacteria in their breast milk. Increasing bacterial counts did not affect the clinical manifestation of mastitis; thus results from bacterial culture of breast milk may be difficult to interpret and bacterial counts in breast milk may therefore be of limited value in the decision to treat with antibiotics. These results suggest that the division of mastitis into infective or non-infective forms may not be practically feasible. Daily follow-up to measure the subsidence of symptoms can help detect those in need of antibiotics.

# Induction of anti-secretory factor in human milk may prevent mastitis

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## **Aim**

The aim of the study was to try to induce anti-secretory factor (AF) in human milk and possibly prevent mastitis.

## **Methods**

Forty mothers who had normal deliveries and healthy full-term infants were randomly divided into two groups, 3-7 days postpartum. The experimental group received a food inducing AF. The control group received the same type of food, without AF-inducing properties. Milk was tested for AF after the mothers had eaten the cereals for 4-5 wk. AF was determined by intravenous injection of milk samples into rats measuring their capacity to prevent secretion into a gut loop of the rat injected with cholera toxin.

## **Results**

The median levels of AF differed between the experimental (n = 12) and control groups (n = 16): 1.1 (0.7-1.25) units vs 0.1 (0.0-0.25) units,  $Z = -4.492$ ,  $p < 0.0001$  (11 mothers dropped out and one milk sample is missing from one of the control mothers). The frequency of mastitis in the experimental compared with the control group was reduced ( $p = 0.0086$ , permutation test). The median AF levels in mothers with or without mastitis differed; 0.0 (0.0-0.1) vs 0.5 (0.2-1.1),  $Z = -2.399$ ,  $p = 0.017$ .

## **Conclusion**

We suggest a specially treated cereal induces AF in human milk and protects against clinically manifested mastitis.

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# Innate immune response in experimental coagulase-negative *Staphylococcus* mastitis

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## Introduction

The role of CNS strains in bovine mastitis has expanded during past years. In Finland 24% of subclinical and 18% of clinical cases are caused by CNS. While the host responses in *S. aureus* mastitis are well characterized, little information exists on the host responses against CNS strains. Pro-inflammatory cytokines are central signalling molecules in infections initiating a cascade of innate immunity events. Their kinetics reflects the disease severity and host's innate immune response.

## Aim

Our aim was to investigate responses of IL-1 $\beta$ , TNF- $\alpha$  and IL-8 in experimental CNS mastitis.

## Material and Methods

The samples were collected from 8 cows, which were infected with two CNS strains (*S. simulans* and *S. epidermidis*). Milk samples from control and infected quarters, bacteriological and clinical data were collected between day -7 and +14. The concentrations of IL-1 $\beta$ , TNF- $\alpha$  and IL-8 were determined by ELISA. The animal experiments were approved by the local animal welfare authorities.

## Results

Viable bacteria were recovered from all four quarters infected. Our preliminary results indicate that milk cytokine levels (IL-1 $\beta$ , TNF- $\alpha$  and IL-8) were typically slightly increased in infected quarters after 12 hours and returned to the background levels by 45 hours.

## Discussion and Conclusions

Elevated cytokine levels indicate an active innate response. Milk IL-8 concentration was significantly lower whereas IL-1 $\beta$  and TNF- $\alpha$  concentrations were at the same level than in coliform, *S. aureus* or streptococcal mastitis. The differences in cytokine profiles reflect the differential host responses to the various microbes and could explain some variations in the clinical signs of the respective infections.

# Acute phase proteins in milk; biomarkers for sub-clinical mastitis and milk quality?

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## Introduction

Mastitis is a major factor influencing milk quality due to the influx of serum components and negative effects on the synthesis of milk components. Also sub-clinical mastitis will induce unfavourable changes in the milk and it is of great importance to identify specific and sensitive biomarkers for rapid detection of affected cows. Milk somatic cell count (SCC) is used both in mastitis diagnosis and as an indirect indicator of milk quality in bulk tank milk. Since SCC is influenced by several physiological factors, much effort has been invested to find alternative biomarkers. During recent years, the potential in mastitis diagnostics of the two major bovine acute phase proteins (APP) haptoglobin (Hp) and serum amyloid A (SAA) has been studied.

## Aim

The overall aim of our work was to study if APP can be used as biomarkers for raw milk quality in relation to udder health.

## Materials and methods

Cow composite samples from clinically healthy cows (n=89) and bulk tank milk samples (n=91) were collected. Hp, SAA, SCC, total protein, casein, whey proteins, proteolysis, fat, and lactose were analysed in all milk samples.

## Results

The composition of milk samples with detectable levels of APP differed significantly from samples without detectable levels of APP, specifically in milk protein. Milk samples with detectable APP contained less of the valuable casein, more whey proteins and more protein was degraded.

## Conclusions

APP are suggested as potential biomarkers for the composition of the milk. In these studies the presence of APP in milk was related to deteriorated protein quality which is of great importance for the dairy industry.

# Peptidomics of high cell count and mastitic milk

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## Introduction

At elevated cell count and mastitis an increased proteolysis of the caseins is often observed compared with normal milk. This is the result of an elevated level of proteolytic enzymes present in the milk. New proteomic and peptidomic methods are now available, permitting the separation and subsequent identification of peptides present in milk by mass spectrometry. By identification of cleavage sites in caseins, from where the peptides originated, it is possible to suggest responsible proteases for the deterioration of milk quality.

## Aim

To identify peptides in milk with different levels of somatic cells and different types of mastitis.

## Materials and methods

The peptide fractions were isolated from the milk samples, and separated by chromatographic methods. The peptides were then identified by MALDI TOF MS<sup>2</sup>.

## Results

An increasing level and number of peptides were seen at elevated cell count and at mastitis. A range of the peptides were identified by mass spectrometry, and the position from where they were derived in the caseins was determined. Based on earlier information from model studies possible responsible proteases were suggested for some of the cleavages.

## Discussion

The studies showed that proteases derived from the somatic cells were potentially responsible for some of the cleavage sites, but also that hitherto unknown proteases may contribute to the observed proteolysis.

## Conclusions

Many peptides are present in milk at elevated cell count and at mastitis. A peptidomic approach involving chromatographic separation combined with mass spectrometry is a useful tool for understanding and explaining the proteolysis that have occurred in the raw milk.

# Is vasopressin involved in milk synthesis and milk ejection?

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## **Introduction**

Suckling increases plasma levels of both vasopressin and oxytocin and their receptors. Intravenous infusions of vasopressin increased milk flow and milk fat content in goats, which could be a result of vasopressin binding to the oxytocin receptor.

## **Aim**

The aim of the present study was to investigate if injection of vasopressin causes milk ejection in the goat.

## **Material and methods**

During an adaptation period of 3 weeks, 8 lactating goats and their kids were separated between 6.30 h – 14.00 h and hand milked twice daily. In total the experimental period consisted of ten control days and two treatment days when the goats were either injected with vasopressin or oxytocin in a crossover design. On treatment days, the goats were hand milked to empty the udder, and then vasopressin or oxytocin was injected into the jugular vein. Two minutes after the injection the goats were milked again to determine the effect of the injection on emptying of residual milk.

## **Results**

The cisternal milk volume ( $85 \pm 3$  g) was similar between treatment days. In control milkings the residual milk volume was  $12 \pm 7$  g, after oxytocin injections  $118 \pm 36$  g ( $p < 0.001$ ) and after vasopressin injections  $36 \pm 18$  g ( $p < 0.01$ ). The fat content was significantly elevated in the residual milk after oxytocin injections ( $3.0 \pm 1.0$  %) compared to vasopressin injections ( $2.4 \pm 0.6$  %) and control milkings ( $2.2 \pm 0.5$  %).

## **Discussion and conclusions**

Vasopressin injections did not cause a milk ejection reflex similar to the oxytocin injections but the residual milk amount was doubled compared to control days. This is consistent with the idea that vasopressin may bind to the oxytocin receptor, but with less efficiency than oxytocin itself.

# Milk composition in udder quarters with different levels of SCC

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## Introduction

A topical question in the dairy milk production is the definition of abnormal and normal milk and which criteria should be used to separate milk. Today automatic milking systems, where quarter milking is used, give an opportunity to separate milk at quarter level. To perform this, increased knowledge about the milk composition alterations due to high SCC (somatic cell count) in separate udder quarters is needed.

## Aim

The aim of the present study was to perform a detailed evaluation of the milk composition from cows with different degree of SCC in separate udder quarters.

## Material and Methods

Quarter milk samples and representative cow composite milk samples were collected from 17 cows divided into three groups. The groups were as follows; group 1: all udder quarters with SCC <50,000 cells/ml, group 2: one udder quarter with SCC 101,000-600,000 cells/ml and the other three udder quarters with SCC <100,000 cells/ml and group 3: one udder quarter with SCC >700,000 cells/ml and the other three udder quarters with SCC <100,000 cells/ml. The milk samples were analyzed for SCC, fat, total protein, lactose, citric acid, whey proteins, casein, non-protein-nitrogen, lactoferrin, protein profile, free fatty acids, LDH, proteolysis, sodium, potassium and milk yield at udder quarter level were registered. Bacteriological samples were collected twice in all cows for all quarters.

## Conclusion

The major conclusion in this study was that we found changed milk composition in separate udder quarters from cows which had composite milk with a low level of SCC, indicating the importance to separate milk at udder quarter level.

## **Early development of breastfeeding competence in preterm infants**

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### **Background**

Breastmilk feeding has particular advantages for preterm infants: reduced risk of severe neonatal infections and improved psychomotor development. An immature sucking behaviour is considered a barrier in the establishment of breastfeeding in these infants.

### **Aim**

To explore the development of breastfeeding capacity in preterm infants.

### **Subjects and methods**

Mothers of (a) preterm (GA < 37 w, n = 71) and (b) very preterm (GA < 32 weeks, n = 15) infants assessed their infants' oral motor behaviour in hospital. Semi-demand feeding: prescription of a total daily volume of milk, and unregulated, frequent breastfeeding was practiced during the transition from scheduled to demand feeding.

### **Results**

Breastfeeding was initiated when the infants did not need assisted ventilation, from 27 w. Rooting was noted from 27 w, obvious rooting, efficient areolar grasp, and staying fixed at the breast for  $\geq 15$  min. from 28 w. Sucking was noted from 26 w, repeated long sucking bursts ( $\geq 10$  sucks) from 29 weeks, and bursts of  $> 30$  sucks from 32 w. Swallowing was perceived from 30 w. Exclusive breastfeeding was attained from 32 w. Intake pattern (frequency, median and maximum volumes) showed a very wide variation.

The infants' weight gain when exclusively breastfed was considered adequate.

### **Discussion**

This early competence can be interpreted as an expression of a survival mechanism in mammals that depends on the mother's presence and sensitivity to the infant's behaviour.

### **Conclusion**

Even very preterm infants have oral motor competence, sufficient for establishment of full breastfeeding at a low postmenstrual age.



# Genetic Analyses of Pathogen-Specific Mastitis

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## Introduction

Mastitis is one of the major health problems in dairy cattle. One approach to lower the frequency is to improve the natural genetic resistance of cows to udder pathogens.

## Aim

To study the presence of genetic variation for resistance to pathogen-specific mastitis in dairy cattle, and to examine whether haplotypes of an identified quantitative trait locus (QTL) with effect on overall mastitis resistance has different effects on specific mastitis pathogens.

## Materials and method

Field data on bacterial diagnoses of mainly subclinical mastitis, collected 1993-2004, were used. After including only daughters of sires with  $\geq 20$  progeny in the data set, it comprised 21,834 cows with 38,607 diagnoses. Variance components were estimated for incidence of the six most frequent pathogens (*S. aureus* 35%, coagulase negative staphylococci (CNS) 29%, *Str. uberis* 13%, *Str. dysgalactiae* 11%, *E. coli* 4%, other streptococci 2%). Pathogen data were scored as a categorical trait with 1 (present) or 0 (absent) for each pathogen at each observation.

## Results

Genetic variation for pathogen-specific mastitis was generally higher compared to corresponding literature estimates on general resistance to clinical mastitis. Estimated genetic variation on the liability scale for acquired infections ranged from 0.006 (*E. coli*) to 0.047 (CNS). To examine whether haplotypes of a previously identified QTL with effect on mastitis resistance had different effects on specific mastitis pathogens, data on 114 genotyped bulls with  $\geq 5$  daughters with bacteriological data were analysed.

## Conclusion

Although there were no haplotype substitution effects on the resistance to any of the 6 mastitis pathogens, two of the haplotypes differed regarding the risk of acquiring a *Str. dysgalactiae* infection.

# Is there a special mechanism behind the changes in somatic cell and polymorphonuclear leukocyte counts, and composition of milk after a single prolonged milking interval in cows?

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## Introduction

A single prolonged milking interval (PMI) is associated with a subsequent short-lasting increase in milk somatic cell count (SCC) which might result in milk quality complaints. The mechanism behind the SCC peak and how milk quality is influenced is poorly investigated.

## Aim

The aim was to investigate the characteristics of the SCC peaks and how milk composition/quality is influenced by a single PMI.

## Material and methods

Composite cow milk samples were taken at the milkings twice daily during 7 days before and 5 days after a PMI of 24 h. Milk was analyzed for SCC, polymorphonuclear leukocytes (PMN) and milk composition.

## Results

Already during the PMI the proportion of PMN (pPMN) increased notably in spite of marginally increased SCC. The peak SCC was not observed until the second milking after the PMI, in afternoon milk. The peak SCC value in *morning* milk did not occur until one day later, surprisingly concomitantly with a *decrease* in the pPMN. After declining, SCC still remained increased while pPMN was *decreased* throughout the study as was also the milk yield. Milk composition was changed the first day after the PMI but the changes were thereafter not consistent except for lactose that remained decreased throughout the study.

## Discussion

It is notable that in several respects, the pPMN after the PMI did not follow the SCC in the way commonly seen during mastitic as well as physiological conditions. At a certain time point SCC appeared to increase due to enhanced recruitment of mononuclear leukocytes, not PMN. There was no indication of capillary leakage or tissue cell damage as possible sources of chemoattractants and in a recent, yet un-published, study no increase in e.g. IL1 $\beta$  was observed after a PMI.

## Conclusions

Our results indicate a special chemotactic background to the recruitment of leukocytes to milk after a single PMI and that milk quality is not impaired.

# **Evaluation of a new mid-infrared transmission spectroscopy instrument for measurement of milk composition in human and buffalo milk**

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Mid-infrared (MIR) analytical instruments have been used for several decades and are today accepted in official laboratory methods for measuring the composition of cow milk and dairy milks. The methods are approved as official method for cow and dairy milk (AOAC, IDF, ISO). The MIR based methods are also appropriate for analysis of milk from other mammals as well as humans. However, there are no official methods for measuring the composition of human milk. Therefore, you will find human milk composition being analyzed with analytical variety of methods. It is very important when making evaluations of new methods that comparisons are performed against official reference methods.

The aim of the two studies were to evaluate a small, low-cost, low volume and portable instrument (Dairy Milk Analyzer (DMA) and Human Milk Analyzer (HMA), Miris AB, Uppsala, Sweden) using mid-infrared transmission. The evaluation was performed at the University Hospital in Lund, Sweden and Chitale Dairy in Pune, India.

The human milk determinations were compared to the ISO official reference methods for cow milk, milk fat (Röse-Gottlieb), milk protein (Kjeldahl) and carbohydrates (Luff-Schoorl). The buffalo milk determinations were compared against the Indian national methods, Gerber (milk fat) and solid-non-fat (Lactometer).

Both studies showed very good performance and high accuracy, comparable to evaluations performed on cow and dairy milk with commercial MIR milk analyzers.

# Evaluation of Factors Affecting Reproductive Performance and Somatic Cell Count in Lithuanian Dairy Cattle Herds

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## **Introduction**

It has long been known that farming manner has impacts on dairy animal performance measures including growth, health, reproduction, and lactation.

## **Aim**

The objective of this study was to evaluate the relationships between herd size, reproductive performance and somatic cell count in Lithuanian Red (R) and Lithuanian Black and White (BW) cattle herds.

## **Material and Methods**

Analysis included 1 297,504 records obtained from a certified recording system. Reproductive indexes - age at first calving (CA, d) and calving interval (CI, d), and somatic cell count (log, SCC) were included into analysis as variables. Models for reproductive indexes and SCC were significant and included, as independent variables, - breed (R, BW), parity (1-16), herd size (1-5; 6-10; 11-50; >50 cows/herd); sampling season (winter, spring, summer, and fall), and CA (<450; 450-900; >900 d) and CI (<270; 270-600; >600 d) – only for SCC.

## **Results**

Breed has significant effect on reproductive indexes but none on SCC. Breed differences for CA and CI were evident for all parities; 834 and 403 for R to 828 and 406 for BW, respectively. Heifers calved at younger ages and later had shorter calving intervals and lower SCC in commercial farms (>50 cows/herd). Mean SCC was lower for parities 1 through 4, and SCC decreased in winter and spring; short CI and elder CA had an impact on increased SCC.

## **Conclusion**

Our investigation indicated better reproductive performance and lower SCC in large commercial farms.

Key Words: cow • dairy • somatic cell count • reproduction • herd size

# **Effect of increased unilateral milking frequency on milk fat and milk protein.**

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## **Introduction**

Increased milking frequency is known to influence milk yield and milk composition. Undesirable effects on milk fat, such as enhanced amount of free fatty acids (FFA) and for milk protein desired effects like lower plasmin activity has been observed. The physiological mechanism behind these effects is not fully understood.

## **Material and methods**

A unilateral milking experiment was performed with eleven cows participating in a twelve day long study with two five day long periods. In the first period the cows were milked on each udder half 2x daily with twelve hour interval, while in the second period two they were milked 2x daily on one udder half and 4x daily with six hour interval on the contralateral udder half. Milk yield was registered, milk samples were analysed for content of fat, FFA, fatty acid composition, fat globule size, content of protein, whey and casein, plasmin and plasminogen-derived activity, and content of Na and K ions.

## **Results and discussion**

In 4x milked udder half the expected increase in milk yield was observed, FFA increased significantly in milk stored cold for 24 hours and the average fat globule size increased. 4x milking had no effect on fatty acid composition and  $\gamma$ -glutamyl transpeptidase. As the average fat globule size became greater due to 4x milking, possibly that contributes to the increased FFA since big fat globules are more susceptible to lipolysis than the small ones. The results indicate that the increased FFA content with 4x milking could not be explained by greater synthesis of short chained fatty acids or insufficient milk fat globule membrane. The protein content tended to be lower in the 4x milked udder half and the plasmin activity decreased. No effects were observed in the level of proteolytic degradation of the milk proteins. Concentration of Na decreased while the K concentration increased. The results indicate that increased milking frequency is favourable for milk protein since the plasmin activity in the more frequently milked glands was significantly lower. The lower levels of Na and higher levels of K in those glands indicate better tight junction integrity with short milking intervals.

## **Conclusion**

With the use of the method unilateral milking, the effects of frequent milking on milk synthesis seems to mainly be regulated locally in the mammary gland, but more studies are needed for a better understanding of the regulatory mechanisms of milk synthesis.

# **Effect of milking frequency on lactation production in an automated milking system**

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## **Introduction**

The lactation persistency is partly controlled by reduced apoptosis (cell death) in early lactation which can be influenced by increased milking frequency before peak lactation. In systems provided with automated milking (AM) where the cows have the possibility to "voluntary" choose milking frequency it is likely that cows with more frequent milking during the first ten weeks of lactation also have a more optimal lactation persistency.

## **Material and method**

Effect of milking frequency on lactation was studied in the AM-system at Kungsängens's research farm, Swedish University of Agricultural Sciences. Lactation curves were studied for SRB cows (Swedish Red Breed), 58 in first lactation and 56 in 2<sup>nd</sup> or 3<sup>rd</sup> lactation. The cows were divided in two groups based upon average milking frequency the first ten weeks, 2.5 and 3.1 milkings respectively. The criteria for cows included in the calculations were that they should be lactating at least 30 weeks and being pregnant.

## **Results and discussion**

Results showed that cows with the highest milking frequency had a significant higher lactation production. The effect was most pronounced in primiparous cows who increased lactation yield by 10.5 % but no effect was observed for milk composition and milk somatic cell count (SCC). The cows in second and third lactation increased their production with 6.0 % and a tendency for a decrease in fat, protein, lactose and SCC was observed. The reason why the response was higher on first calvers could be an effect of the udder anatomy, where young cows have less cisternal fraction compared to older cows.

## **Conclusion**

A higher milking frequency than two times a day in beginning of lactation seems to increase production capacity during entire lactation. With AM system it is possible to control the milking frequency in the beginning of lactation.

# Somatic cell count and quality of milk during pasture turnout of dairy cows

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## Introduction

Milk somatic cell count (SCC) is a predominant criterion for milk quality since it has been observed that high SCC decreases the milk quality. The SCC is known to increase during the grazing season but the question is, does this increase affect the milk quality?

## Aim

The aim of the study was to investigate if there is a spontaneous increase in SCC when cows are let out on pasture, and if so whether it has an effect on milk quality.

## Materials and methods

A total of 35 dairy cows were included in the study carried out at Kungsängen Research Centre, SLU, Uppsala. The cows were in lactation day 12–442, lactation no 1–5. The cows were machine milked twice a day, at 06.30 and 16.00. Sampling was conducted during seven days prior to, and during five days after pasture turnout. Milk samples were collected for analyses of the milk composition, SCC and PMN (polymorphonuclear leukocytes). For the afternoon milking, samples were also collected for analyses of Free Fatty Acids (FFA) and casein.

## Results

The SCC and PMN increased following pasture turnout for both morning and afternoon milking. There was a significant drop in milk yield during the morning milking Day 1 compared with the baseline value but no drop during the afternoon milking. From Day 3, the milk yield instead increased significantly during both morning and afternoon milking compared with the baseline value. The concentration of fat increased all days compared with the baseline value whereas for the afternoon, fat decreased Day 0 and then increased Days 2 and 3 to a level similar to the baseline. The FFA level was significantly lower during the afternoon milking Day 1 compared with Day -1. The protein increased all days compared with the baseline value during the morning milking but decreased Day 0 and Day 1 during the afternoon milking. The casein level increased Day 3 and Day 5 compared with Day -1 whereas they did not change due to pasture let-out.

## Conclusions

In conclusion, there was a marked peak in milk SCC and PMN in response to pasture turnout. However, the milk quality was not affected by the short SCC peak and one explanation could be the fairly low level of SCC even with a marked peak.





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