# Lactation Biology Symposium: Mammary gland biology revisited

**95 Mammary gland growth—It's not just estrogen.** Russell C. Hovey*[1], Grace E. Berryhill[1], Josephine F. Trott[1], and Adam L. Lock[2], *[1]University of California, Davis, Davis, CA, [2]Michigan State University, East Lansing, MI.*

The mammary gland goes through a coordinated series of developmental states during postnatal life in preparation for lactation. These changes ultimately reflect a changing endocrine environment alongside local alterations in the microenvironment. A longstanding assumption has been that estrogens serve as the first essential component for any of these developmental changes to be realized in the mammary glands. Indeed, estrogens can initiate the onset of allometric growth during puberty, and they subsequently potentiate the effects of other endocrine cues on the mammary epithelium during lobulo-alveolar development throughout gestation. More recent evidence from our laboratory indicates that alternative pathways are equally effective in promoting growth of the mammary glands independent of a primary role for estrogens. One fascinating example is the ability of dietary *trans*-10,*cis*-12 conjugated linoleic acid (10,12 CLA) to promote allometric growth of the mammary ducts of ovariectomized mice. In subsequent studies we showed that the specific effects of this dietary fat are independent of estrogenic action, and are mediated through IGF-I receptor signaling. The relevance of these findings is emphasized by the fact that this dietary intervention mimics several aspects of the metabolic syndrome manifest in obese humans. At the same time, additional findings from our laboratory indicate that prolactin and progesterone have synergistic effects on epithelial growth and morphogenesis independent of any role for estrogens, which are partly mediated by changes in the local microenvironment. These data collectively support the notion that while estrogens are clearly mitogenic for the growing and developing mammary glands, they are not necessarily requisite. These findings have potential implications for our understanding of mammary growth in all mammals including livestock, as well as direct relevance to the regulation of mammary cancers.

**Key Words:** prolactin, progesterone, lipid

**96 Body condition of gilts at the end of gestation affects their mammary development.** Chantal Farmer*[1], Marie-France Palin[1], and Michel Vignola[2], *[1]Agriculture and Agri-Food Canada, Dairy and Swine R&D Centre, Sherbrooke, QC, Canada, [2]Nutreco Canada, St-Elzéar, QC, Canada.*

The goal of this project was to determine if changes in body condition that incur during gestation affect mammary development of gilts on d 110 of gestation. Gilts of a similar BW (138.1 ± 8.2 kg) and backfat thickness (BF, 16.4 ± 1.0 mm) at mating were fed different amounts of a commercial gestation diet to create 3 groups of animals based on body condition at the end of gestation. These were low (LO; 12–15 mm, n = 13), medium (ME; 17–19 mm, n = 13), and high (HI; 21–26 mm, n = 13) BF. All gilts were weighed and had their BF measured ultrasonically at P2 the day of mating and on d 30, 50, 100 and 110 of gestation. Blood samples were obtained on d 109 of gestation to measure IGF-1 and adiponectin concentrations. Gilts were then slaughtered on d 110 to collect mammary glands for compositional analyses. The MIXED procedure of SAS using a univariate model (3 levels) was used for statistical analyses and means were compared using the Tukey's test. As expected, BW and BF were similar across all groups at mating ($P > 0.10$). Treatment differences were present for both variables as of 30 d of gestation onward ($P < 0.01$). Neither IGF-I nor adiponectin concentrations were affected by treatment ($P > 0.10$). Mammary extraparenchymal tissue weight was lesser in LO than in ME or HI gilts (1074.7, 1360.2 and 1578.4 ± 64.3 g, respectively, $P < 0.01$) and tended to be lesser in ME than in HI gilts ($P = 0.06$). Weight of parenchymal tissue was also affected by treatment ($P < 0.05$), being lesser in LO than HI gilts ($P < 0.05$), and tending to be lower in LO than ME gilts ($P = 0.12$). Values for LO, ME and HI gilts were 1058.6, 1369.6 and 1443.9 ± 198.7 g, respectively. Mammary cell number (DNA) in parenchyma was not affected by treatment, whereas metabolic activity (RNA) was greater in LO than HI gilts ($P < 0.05$). Total parenchymal fat was also lesser in LO than ME or HI gilts ($P < 0.001$). Body condition of gilts therefore has an impact on mammary development at the end of gestation. Thanks to Swine Innovation Pork for partial funding.

**Key Words:** backfat thickness, gilt, mammary development

**97 Autocrine-paracrine regulation of the mammary gland.** Samantha R. Weaver and Laura L. Hernandez*, *University of Wisconsin-Madison, Madison, WI.*

The mammary gland has a remarkable capacity for regulation at a local level, particularly with respect to its main function: milk secretion. Regulation of milk synthesis has significant affects animal and human health, at the level of both the mother and the neonate. Control by the mammary gland of its essential function, milk synthesis, is an evolutionary necessity and is therefore tightly controlled at a local level. For at least the last 60 years, researchers have been interested in elucidating the mechanisms underpinning the mammary gland's ability to self-regulate, largely without the influence from systemic hormones or signals. By the 1960s, researchers realized the importance of milk removal in the capacity of the gland to produce milk and that the dynamics of this removal, including emptying of the alveolar spaces and frequency of milking, were controlled locally as opposed to through systemic hormonal regulation. Using both in vitro systems and various mammalian species, including goats, marsupials, humans, and dairy cows, it has been demonstrated that the mammary gland is largely self-regulating in its capacity to support the young, which is the evolutionary basis for milk production. Local control occurs at the level of the mammary epithelial cell through pressure and stretching negative-feedback mechanisms and also in an autocrine fashion through bioactive factors within the milk which act as inhibitors, regulating milk secretion within the alveoli themselves. It is only within the last 20 to 30 years that potential candidates for these bioactive factors have been examined at a molecular level. Several factors, including parathyroid hormone related protein (PTHrP), growth factors (transforming growth factor, insulin-like growth factor, epidermal growth factor) and serotonin (5-HT), are both synthesized within and act upon the gland, and posess dynamic receptor activity resulting in diverse effects on growth, calcium homeostasis, and milk composition. This review will focus on the autocrine-paracrine regulation of the mammary gland, with an examination of both foundational work and the progress made within the last 10 to 20 years of research.

**Key Words:** lactation, milk, secretion

**98 New insights in the importance of prolactin in dairy ruminants.** Pierre Lacasse*[1], Séverine Ollier[1], Vanessa Lollivier[2], and Marion Boutinaud[2], *[1]Dairy and Swine R&D Centre, Sherbrooke, QC, Canada, [2]INRA, Agrocampus Ouest, UMR1348 PEGASE, Saint Gilles, France.*

In most mammals, prolactin (PRL) is essential for maintaining lactation and its suppression inhibits lactation. However, the involvement of PRL in the control of ruminant lactation is less clear because inconsistent effects on milk yield have been observed with short-term suppression of PRL by bromocriptine. Therefore, several experiments were conducted to assess the galactopoietic role of PRL. In an initial experiment, cows in early lactation received daily injections of the dopamine agonist quinagolide (QUIN) for 9 weeks. QUIN reduced milking-induced PRL release and caused a faster decline in milk production. Milk production was correlated with the amount of PRL released at milking. QUIN reduced mammary epithelial cell activity, survival and proliferation. In goats, QUIN did not affect either basal or milking induced PRL release and milk production, whereas injection of cabergoline, another dopamine agonist, caused a decrease of 28% of milk yield the day after the injection. In another experiment, cows were injected for 5 d with QUIN; QUIN + injection of bPRL at milking time; or vehicles. Again, milk, protein and lactose yield were decreased by QUIN. Although PRL injections were not sufficient to restore milk yield, they tended to increase milk protein and lactose yields and increased the viability of milk purified mammary epithelial cells. In late lactation cows, QUIN decreased milk production within the first day of treatment and induced a more rapid changes in several markers of mammary gland involution after drying-off. Similarly, injection at drying-off of cabergoline hastened mammary involution and enhanced mammary gland remodeling. Recently, we stimulated PRL secretion with daily injection of the dopamine antagonist domperidone for 5 weeks. Milk production increased gradually and was greater in domperidone-treated cows during the last 4 weeks of the treatment period. Milk production of both groups became similar again 5d after the last injection. In conclusion, these data, combined with those from other studies, provide a good body of evidence that PRL is galactopoietic in dairy ruminants.

**Key Words:** prolactin, milk production, cows

**99 Regulation of cell number in the mammary gland via the control of the exfoliation process in milk in ruminants.** Lucile Hervé[1,2], Vanessa Lollivier[1,2], Hélène Quesnel[1,2], and Marion Boutinaud*[1,2], *[1]INRA UMR1348, Saint Gilles, France, [2]Agrocampus Ouest UMR1348, Rennes, France.*

Milk yield is partly influenced by the number of mammary epithelial cells (MEC) in the mammary gland. It is well known that MEC number varies due to cell proliferation and apoptosis. The exfoliation of MEC from the mammary epithelium into milk is another process which might influence MEC number in the mammary tissue. Yet, little is known about the control of MEC exfoliation process. The rate of MEC exfoliation can be assessed by measuring the milk MEC content through flow cytometry analysis or through an immuno-magnetic method for MEC purification. Various experimental models were used to affect milk yield and study the rate of MEC exfoliation. Reducing milking frequency from twice to once daily increased MEC loss per day in goat but not in cow milk. An increased daily rate of MEC exfoliation was also observed during short days as compared with long days or in response to an endotoxin-induced mastitis in cows. Other animal models were designed to investigate the endocrine control of the exfoliation process and its link with milk production. Suppression of ovarian steroids by ovariectomy resulted in a greater persistency of lactation and a decrease in MEC exfoliation. Administering prolactin inhibitors enhanced MEC loss while exogenous prolactin tended to prevent this negative effect of prolactin inhibitors. These findings suggest that prolactin could regulate MEC exfoliation. In most of these studies, variations of MEC exfoliation were associated with variations in milk yield and changes in mammary epithelium integrity. Exfoliation of MEC could be a process that regulates MEC number in the mammary tissue, and thereby could influence milk yield and lactation persistency.

**Key Words:** cow, lactation, mammary epithelial cell

**100 Mammary response to infection: A critical balance between pathogen elimination and collateral damage.** David E. Kerr*, *University of Vermont, Burlington, VT.*

Mastitis is an inflammatory disease of the mammary gland. The disease is generally classified as sub-clinical (no obvious signs) or 3 levels of clinical disease including: mild (abnormal milk); moderate (abnormal milk with swelling or redness of the gland); severe (abnormal milk, gland inflammation, with systemic signs of illness). Recent large-scale studies indicate that mild, moderate, and severe forms make up approximately 60%, 30%, and 10% of clinical cases of mastitis, with severe mastitis predominately caused by gram-negative bacteria. Efforts to reduce severe mastitis are of utmost importance to dairy animal welfare, and these cases are associated with greatest milk production losses. Experimental challenge studies under controlled conditions reveal animal-to-animal variation in the severity of the resulting mastitis. This suggests a genetic basis to disease severity and the potential for finding genetic markers for use in breeding programs to produce animals with a reduced tendency to develop severe mastitis. However, the evolving field of epigenetics suggests that in utero and early life environments can modify gene expression and thus modify an animal's phenotype. Our approach is to develop a cell culture challenge model predictive of an animal's innate response phenotype. Such a model could potentially be used with cells from young animals to determine their response phenotype and thus facilitate selection of herd replacements. In our dermal fibroblast model, the cells are cultured under controlled conditions and then challenged with LPS to determine innate response magnitude. This model has revealed breed differences (Angus vs. Holstein) and epigenetic differences in samples from the same animals (i.e., same genotype) collected at 5 and 16 mo of age. Further, animals with low vs. high fibroblast response phenotype produce less BSA in milk following experimentally induced mastitis. Future studies employing this and other model systems, combined with well-controlled disease challenges of extreme phenotypes will lead to a greater understanding of factors contributing to animal variation in the severity of response to mammary infection.

**101 Blood-derived proteins in milk during the colostral period: Active or passive transfer?** Samantha K. Wall*[1], Josef J. Gross[1], Evelyne C. Kessler[1], Kris Villez[2], and Rupert M. Bruckmaier[1], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland.*

Colostrum has a different composition than milk in established lactation. This difference is in part due to the partially open blood-milk barrier, which prevents the interdiffusion of blood and milk components. In the first days of lactation, α-lactalbumin (LALBA), a milk protein, is typically present in blood and several blood-derived proteins are present in milk such as $IgG_1$ (very high concentration), $IgG_2$, serum albumin (ALB), and lactate dehydrogenase (LDH). With the exception of $IgG_1$, which is transferred by active transcellular transport, other proteins are

thought to pass paracellularly through the temporarily open barrier. This study aimed to examine the decline patterns of each protein relative to $IgG_1$, to distinguish between paracellular and transcellular transport through the blood-milk barrier during the first days of lactation. Ten Holstein cows were milked at 4 h after parturition, the next 5 consecutive milkings, and the afternoon milking on d 5, 8, 10, and 14 of lactation for a total of 10 milking time points and blood samples were taken in parallel. Blood and milk samples were analyzed for the concentrations of LDH, ALB, $IgG_1$, $IgG_2$ and LALBA. Protein concentration curves were generated from all 10 time points and were evaluated using the tau time constant model to determine the rate of decline of the slope of each protein. When examining blood-derived proteins in milk, the concentration of $IgG_1$ declined significantly faster than the proteins $IgG_2$ and LDH. Interestingly, the decline of ALB was not statistically different from $IgG_1$ nor $IgG_2$ and LDH. $IgG_1$ concentration in milk far exceeded levels in plasma, and this protein exhibited a recovery increase in plasma during the experimental period. $IgG_2$, ALB, and LDH concentrations in milk did not reach plasma levels. Plasma LALBA followed a different pattern, declining significantly slower than all blood-derived proteins in milk. These results indicate that there is active transport of only $IgG_1$, with a sharp decline at parturition, compared with $IgG_2$, ALB, LDH, and LALBA which are following the closure of the blood-milk barrier.

**Key Words:** colostrum, blood-milk barrier, blood-derived protein