Lactation Biology: Local Control of Mammary Function

559 Regulation of gene expression in the bovine mammary gland by ovarian steroids. E. E. Connor*[1], M. J. Meyer[2], R. W. Li[1], M. E. Van Amburgh[2], Y. R. Boisclair[2], and A. V. Capuco[1], [1]*Bovine Functional Genomics Laboratory, USDA-ARS, Beltsville, MD*, [2]*Cornell University, Ithaca, NY*.

It is well established that estrogen is required for mammary epithelial cell proliferation and ductal development in the growing animal, and that lobuloalveolar development during gestation is dependent upon progesterone. Effects of these steroid hormones on gene expression in the mammary gland are mediated primarily by their respective nuclear hormone receptors that function as hormone-bound transcription factors. To gain insight into how estrogen and progesterone regulate mammary gland growth and function in cattle, we and others have characterized the expression patterns of their cognate receptors in bovine mammary gland throughout development, pregnancy and lactation. This work has identified a lack of expression of ER beta and a greater abundance of PR during lactation in bovine mammary gland versus the rodent gland. We speculate that interactions among the ER isoforms that regulate PR expression may contribute to these species differences. Further, demonstrated expression of substantial quantities of ER within the prepubertal bovine mammary fat pad, along with coordinated IGF-1 expression, suggests this tissue may stimulate parenchymal growth via an estrogen-responsive paracrine mechanism. In addition, the recent availability of bovine genomic sequence information and microarray technologies has permitted the study of global gene expression in the mammary gland in response to the steroid environment. We have identified >100 estrogen-responsive genes of which the majority were not previously reported to be estrogen-responsive. Estrogen-induced changes in gene expression were consistent with increased mammary epithelial cell proliferation, increased extracellular matrix (ECM) turnover in parenchyma, and increased ECM deposition in the fat pad. A comparison of estrogen-responsive genes in the mammary glands of humans, mice and cattle suggests considerable variation among species, as well as potential differences in regulatory elements in common ER gene targets. Continuing studies using advanced molecular techniques should assist in elucidating the complex regulation of mammary function at the transcript level.

Key Words: Gene expression, Steroid hormones, Mammary function

560 Dynamics of lactogenic hormone induced recuitment of transacting-factors to a milk protein gene promoter. E. Kabotyanski[1], M. Rijnkels*[2], M. Huetter[1], and J. M. Rosen[1], [1]*Baylor College of Medicine, Houston, TX*, [2]*ARS /USDA Children's Nutrition Research Center, Houston, TX*.

The main goal of this work is to understand the mechanisms by which hormones and growth factors regulate mammary gland development and lactation. We study the mechanisms by which the lactogenic hormones hydrocortisone (HC) and prolactin (P) regulate milk protein gene expression. We investigated the dynamics of assembly of the different transacting-factors and co-activators that mediate beta-casein (b-CSN) expression (CAAT/enhancer binding protein beta (C/EBP-beta), Yin Yang-1 (YY-1), signal transducers and activators of transcription 5 (STAT5), glucocorticoid receptor (GR), and p300) as well as the histone acetylation status at the hormonally induced endogenous b-CSN promoter and enhancer (BCE, -6kb) in murine mammary epithelial cells (HC11), using Chromatin Immuno-Precipitation (ChIP) analysis. P stimulates the recruitment of STAT5 to the promoter and BCE, but HC+P are needed for expression and synergistically increase STAT5 recruitment. P and the recruitment of STAT5 result in the loss of YY1 bound at the promoter correlating with the relief of repression of b-CSN expression. HC stimulates the recruitment of GR to the promoter but is not enough to initiate b-CSN expression. P and STAT5 recruitment enhance GR presence on the promoter and are required for b-CSN expression. Each hormone separately increases recruitment of C/EBP with an additive effect with both hormones. Either hormone recruits P300 with an additive effect with both hormones, correlating with the increase of histone H3 acetylation. RNA polymerase-II is recruited rapidly to the promoter and the appearance of phospho-pol-II correlates with the detection of b-CSN transcripts. These data suggest a model for the assembly of a multi-protein complex at the beta-casein regulatory regions that helps to understand how the signaling pathways regulated by lactogenic hormones and local growth factors are integrated in the nucleus to direct milk protein gene expression.

Key Words: Lactation, Casein, Gene regulation

561 Udder changes and milk production in dairy ewes induced to lactate. B. Ramírez Andrade[1,2], A. A. K. Salama[1], G. Caja*[1], V. Castillo[1], E. Albanell[1], and X. Such[1], [1]*Grup de Recerca en Remugants, Universitat Autònoma de Barcelona, Bellaterra, Spain*, [2]*Facultad de Agronomía, Universidad Autónoma, San Luis Potosí, México*.

A total of 22 ewes (17 nulli- and 5 multi-parous) of 2 breeds differing in milk yield and milkability (MN, Manchega; 62 kg BW, n=8; LC, Lacaune; 64 kg BW, n=14) were used to evaluate a modified protocol for inducing lactation. Dimethyl sulfoxide (DMSO) replaced 96% ethanol as a steroid solvent to avoid the necrotic wounds caused by ethanol in sheep skin. Estrus was synchronized over 12 d using 40 mg FGA sponges and 400 IU PMSG. Induction started 5 d after sponge withdrawal, and consisted of s.c. injection of estradiol (0.5 mg/kg BW) and progesterone (1.25 mg/kg BW) for 7 d, s.c. bST (200 mg) at d 11, and i.m. hydrocortisone acetate (50 mg) from d 18 to 20. Machine milking with hand stimulation and i.v. oxytocin (2 IU) began on d 21. Standard milking routine (x2 daily) was applied from d 4 to 56. Milk was measured and sampled daily during the first 10 DIM and weekly thereafter. Teat length, and udder volume and depth were measured before induction and at 50 DIM. All ewes responded to the induction but 1 MN (5%) yielded <200 mL/d and was excluded. Milk at the first milking (d 21) was similar between breeds (536 ± 130 mL), but multiparous ewes had more milk than nulliparous (834 ± 155 vs 237 ± 98 mL, respectively; $P<0.01$). Milk from 1 DIM was similar in both breeds, mimicked colostrum (fat, 7.48%; protein, 10.2%; whey protein, 6.0%; TS, 21.7%) and decreased to normal values thereafter. Average milk yield for 56 DIM was lower ($P<0.05$) in MN (404 ± 50 mL/d) than in LC (545 ± 45 mL/d). Milk peaked at 35 DIM and tended ($P<0.12$) to be greater in LC (711 ± 57 mL/d) than MN (566 ± 65 mL/d). Teat length increased from 20.5 ± 1.1 mm before treatment to 27.7 ± 1.1 mm at 50 DIM with no differences between breeds. Udder volume (269 ± 50 mL) and udder depth (7.2 ± 1.3 cm) before treatment were similar between breeds. However, at 50 DIM, LC showed greater udder volume (898 ± 63 vs 632 ± 78 mL; $P<0.05$) and tended to have a greater udder depth (18.0 ± 1.5 vs 15.0 ± 1.5 cm; $P<0.14$) than MN ewes. Despite yield and milkability differences, both breeds responded to the DMSO based treatment (95% on average), avoiding the use of ethanol as steroid solvent for lactation induction.

Key Words: Lactation, Milk, Sheep

562 Comparative genomics of the Tammar Wallaby and Fur Seal; model systems to study local regulation of mammary gland function. K. Nicholas*[1,2], M. Digby[1,2], C. Lefevre[1,2], J. Sharp[1,2], S. Mailer[1,2], A. Brennan[1,2], J. Arnould[4], and K. Cane[1,2], [1]*Cooperative Research Centre for Innovative Dairy Products*, *Melbourne, Australia*, [2]*Department of Zoology, University of Melbourne*, *Melbourne, Australia*, [3]*Victorian Bioinformatics Consortium, Monash University*, *Clayton, Australia*, [4]*School of Biological and Chemical Sciences, Deakin University*, *Burwood, VIC, Australia*.

Comparative genomics is providing opportunities to identify key genes regulating mammary gland development, milk production and composition. The application of this technology to species with extreme adaptation to lactation allows the identification and study of regulatory mechanisms that are present but not readily apparent in other species, and secondly allows the identification of novel molecules and processes for application in the biotechnology market. For example, the tammar wallaby has adopted a reproductive strategy that includes a short gestation (26 days), birth of an immature young and a relatively long lactation (300 days). Both the rate of production and the composition of milk change progressively during the lactation cycle to meet the nutritional demands for investment in considerable development of the pouch young (PY) prior to weaning. The lactating mother, not the sucking pattern of the PY, regulates these changes which in turn determines the rate of PY growth and development. Furthermore, the tammar can practice concurrent asynchronous lactation; the mother provides a concentrated milk high in protein and fat for an older animal which is out of the pouch and at heel, and a dilute milk low in fat and protein but high in carbohydrate from an adjacent mammary gland for a newborn pouch young. Our second study species, the fur seal, has a lactation characterized by a repeated cycle of long at-sea foraging trips (up to 28 days) alternating with short suckling periods of 2-3 days ashore. Lactation almost ceases while the seal is off shore but the mammary gland does not progress to apoptosis and involution. Our studies have exploited these models by using microarray analysis and comparative genomics to investigate how mammary function is regulated by endocrine factors, milk and factors intrinsic to the gland.

563 Acute physical distension of rat mammary glands induces apoptosis and decreases β1-integrin and tight junction (TJ) protein signalling. C. V. C. Phyn*[1,2], J. M. Dobson[1], S. R. Davis[3], K. Stelwagen[1], and K. Singh[1], [1]*AgResearch Ltd.*, *Hamilton, New Zealand*, [2]*Dexcel Ltd.*, *Hamilton, New Zealand*, [3]*ViaLactia Biosciences (NZ) Ltd.*, *Auckland, New Zealand*.

The change in epithelial cell shape during mammary engorgement from a cuboidal to a flattened morphology may initiate changes in protein and gene expression (mechanotransduction) causing loss of secretory activity and apoptosis. Down-regulation of β1-integrin and TJ protein expression reflect a loss of cell-extracellular matrix communication and TJ integrity, respectively, during mammary apoptosis. This study examined the effect of acute physical distension of rat mammary glands on apoptosis and the protein expression of β1-integrin and TJ components (occludin, claudin-1 and zonula occludens-1 (ZO-1)). Sprague-Dawley rats at peak lactation each had two teats sealed to induce mammary engorgement. Another gland was acutely distended by infusing 1 ml of isosmotic sucrose solution (≈ 6h of milk secretion) up-the-teat prior to sealing. The remaining unsealed teats served as suckled controls. Mammary tissue was collected post-mortem at 0, 1, 3, and 6h after teat sealing (n=6 per time point). A dramatic increase (P<0.001) in the number of apoptotic nuclei occurred at 1, 3 and 6h in infused glands, and at 6h in engorged glands, compared with controls. By 3h and at 6h, apoptosis in infused glands was also significantly greater than engorged glands. This was accompanied by a reduction in the expression of β1-integrin in infused glands compared with control (P<0.05) and engorged (P<0.1) glands by 6h. Occludin protein expression was significantly up-regulated in infused glands immediately following physical distension at 0h compared with control and engorged glands, but then declined to be down-regulated within 6h of teat-sealing. Claudin-1 and ZO-1 protein expression were significantly decreased in engorged glands, but not infused glands, compared with controls by 6h. These results indicate that acute physical distension of rat mammary glands accelerates the induction of apoptosis and loss of β1-integrin and occludin protein expression compared with milk accumulation alone. In conclusion, we suggest a role for mechanotransduction during apoptosis and involution of mammary glands.

Key Words: Mammary apoptosis, Mechanotransduction, Tight junction

564 Effects of frequent milking during early lactation on milk yield in dairy cows are locally regulated. E. H. Wall* and T. B. McFadden, *Lactation and Mammary Gland Biology Group, University of Vermont*, *Burlington*.

We hypothesized that the effects of frequent milking during early lactation on milk production in dairy cows are regulated via local mechanisms. A unilateral frequent milking model (**UFM**; 2X left side, 4X right side) was used to test our hypothesis. Ten multiparous cows were assigned at parturition to UFM for days 1 to 21 of lactation. After treatment, cows were milked twice daily for the remainder of lactation. At the first milking post-calving, cows were quartermilked to verify that udder halves produced equal amounts of milk prior to treatment. Thereafter, quartermilking was performed on days 3 and 7, then weekly for the first 5 wks of lactation and once every 3 mo for the remainder of lactation. Cows responded quickly to treatment, producing 2 ± .5 kg more milk/d from the 4X side than the 2X side by day 3 (P < 0.01). During UFM, cows produced 3.9 ± .7 kg more milk/d from the 4X side than the 2X side (P < 0.001). Upon cessation of treatment, milk production from the 4X side decreased (P < 0.01), but remained at 1.8 ± .5 kg more milk/d than the 2X side for the remainder of lactation (P < 0.01). It was noteworthy that the difference in milk production between udder halves remained throughout lactation and was still significant at 270 DIM. After correcting milk yield to the equivalent of a whole udder basis, acute milk yield responses to frequent milking were consistent with previous reports. However, we observed greater persistency in the milk yield response, which lasted throughout lactation. We conclude that both immediate and persistent effects on milk production of frequent milking during early lactation are regulated at the level of the mammary gland. Our results also demonstrate that UFM is a valid and efficient model for investigating the effects of frequent milking during early lactation in dairy cows.

Key Words: Dairy cow, Frequent milking, Half-udder

565 Expression and regulation of glucose transporters in the bovine mammary gland. F.-Q. Zhao* and A. F. Keating, *University of Vermont*, *Burlington*.

Glucose is the primary precursor for synthesis of lactose, which controls milk volume by maintaining the osmolarity of milk. Glucose uptake in the mammary gland is considered to be a rate-limiting step

for milk production. Glucose transport across the plasma membranes of mammalian cells is carried out by two distinct processes: facilitative transport which is mediated by a family of facilitative glucose transporters (GLUT) and the sodium dependent transport mediated by the Na+/glucose co-transporters (SGLT). Glucose transport kinetic studies show that glucose transport across the plasma membrane of the lactating mammary epithelial cell has a Km value of 8.29 mM for 3-O-methyl-D-glucose and can be inhibited by both cytochalasin B and phloretin, indicating a facilitative transport process. This is consistent with the observation that in the lactating bovine mammary gland, GLUT1 is the predominant glucose transporter. However, the bovine lactating mammary gland also expresses GLUT3, GLUT4, GLUT5, GLUT8, GLUT12, and sodium dependent SGLT1 and SGLT2 at different levels. Studies of protein expression and cellular and subcellular localizations of these transporters are needed to address their physiological functions in the mammary gland. From late pregnancy to early lactation, expression of GLUT1, GLUT8, GLUT12, SGLT1 and SGLT2 mRNA increases from at least five-fold to several hundred-fold, suggesting that these transporters may be regulated by lactogenic hormones and have roles in milk synthesis. The GLUT1 protein is detected in the lactating mammary epithelial cells with subcellular distribution in both the plasma membrane and cytoplasm. Its level decreases from the early to late lactating stages and becomes barely detectable in the non-lactating stage. Both GLUT1 mRNA and protein levels in the lactating mammary gland are not significantly affected by administration of exogenous bovine growth hormone, and in addition, GLUT1 mRNA expression does not appear to be affected by leptin.

Key Words: Glucose uptake, Milk synthesis, Mammary gland

566 Hormonal interactions between the mammary fat pad and mammary cells affect lactation. Y. Feuermann*[1], S. J. Mabjeesh[2], and A. Shamay[1], [1]*Agriculture Research Organisation The Volcani Center*, *Bet Dagan Israel*, [2]*Faculty of Agriculture, The Hebrew University of Jerusalem*, *Rehovot, Israel*.

Locally produced growth factors are believed to mediate the bovine mammary adipocyte-epithelial cell interactions and alter the actions of several steroid and peptide hormones in these tissues. In a series of in vitro studies, we showed that leptin is secreted from the mammary fat pad, and is regulated by prolactin, the highest level of leptin secretion (16ng/ml) was observed at prolactin concentration of 1µg/ml ($P<0.05$, n=5). The expression of alpha-casein in a co-culture of primary epithelial cells and bovine fat explant was enhanced 4.1 times by prolactin in comparison to epithelial cells cultured alone ($P<0.05$, n=3). Prolactin (1µg/ml) and leptin (100ng/ml) enhanced the expression of StAR mRNA by 2.8 times in the mammary fat but had no significant effect on StAR mRNA expression in primary culture of mammary epithelial cells. The highest level of estrogen receptor mRNA expression in mammary primary culture cells was observed with the combined treatment of prolactin (1µg/ml) and leptin (100ng/ml), the expression was enhanced by 3.1 times ($P<0.05$. n=3) (determined by real time PCR). A significant amount of udder fat was found in calves grown on milk compared to calves grown on milk replacer. Milk-yield of heifers that had high fat udder content (285±27.1g, n=6) was higher than heifers that had low fat udder content (151±11.6g, n=6), (37.7Kg/per day versus 33.6Kg/per day respectively, $P<0.003$). We believe that the difference between the groups was a result of the fat content of the udders, which directly affected the local leptin secretion. Based on the results from the in-vivo and the in vitro experiments, we hypothesize that the amount of fat in the udder affected the level of local leptin and estrogen secretion, the interaction between leptin, which is secreted from the mammary fat pad and prolactin affects the expression of estrogen receptor in the mammary cells.

Key Words: Leptin, Estrogen receptor, Prolactin

567 Growth hormone stimulates the expression of milk protein genes in bovine mammary epithelial cells overexpressing growth hormone receptor. Y. Zhou*, R. M. Akers, and H. Jiang, *Virginia Tech*, *Blacksburg*.

Growth hormone (GH) can increase milk production in cattle and this effect is widely believed to be mediated by indirect action of GH on the mammary gland. However, recent findings that both GH receptor (GHR) mRNA and protein are expressed in the epithelial cells of the bovine mammary gland suggest that GH may have a direct effect on the milk-producing cells. The objective of this study was to determine whether GH could affect milk protein gene expression, nutrient transport and proliferation of the bovine mammary epithelial cells. The bovine mammary epithelia-derived cell line MAC-T cells did not express detectable GHR (perhaps as a result of immortalization) and they were rendered GH responsive by GHR overexpression. Growth hormone treatment of these cells markedly increased ($P<0.01$) the expression of α_{s1}-casein, α_{s2}-casein, β-casein and α-lactalbumin mRNA, whereas it had no effect ($P>0.05$) on the expression of κ-casein, β-lactoglobulin, insulin-like growth factor I (IGF-I), IGF-II or IGF binding protein-1 (IGFBP-1) to IGFBP-6 mRNA. Growth hormone also had no effect on glucose, amino acid or fatty acid transport into, or the proliferation of, these cells. A sequence analysis revealed that the promoters of the bovine α_{s1}-casein, α_{s2}-casein, β-casein and α-lactalbumin genes contain putative binding sites for signal transducer and activator of transcription 5 (STAT5). Cotransfection analyses confirmed that GH could stimulate ($P<0.05$) reporter gene expression from these promoters in MAC-T cells. These in vitro observations, together with the fact that GH receptor mRNA and protein are expressed in the epithelial cells of the bovine mammary gland, suggest that GH may directly stimulate transcription of major milk protein genes through STAT5 in the mammary gland, thereby increasing milk production in cattle.

Key Words: Growth hormone, Cattle, Milk production