M133 Nursing frequency alters circadian patterns of mammary gene expression in lactating mice. D. L. Hadsell,* W. Olea, and L. W. Rottman, *Baylor College of Medicine, Houston, TX.*

Milking frequency impacts lactation in dairy cattle and in rodent models of lactation. The role of circadian gene expression in this process is unknown. The hypothesis tested was that changing nursing frequency alters the circadian patterns of mammary gene expression. Mid-lactation CD1 mice were studied in 2 groups, for up to 54 h. Ad-libitum (AL) nursed dams were allowed AL access to their litters for the entire study. Variably nursed (VN) dams were placed on a 4-time (4X) per day (d) schedule on d1, and an AL nursing schedule on d 2 of the study. Samples were collected at 6 h intervals (n = 3 dams/ treatment-time combination). Mammary gland (MG) weight was used to indicate the combined effects of secretory activity and suckling-dependent milk removal. Total MG RNA was analyzed by real-time qRT-PCR for the expression of Bmal, Per1, Per2, Lalba, and B4galt1. On d 1, MG weight in the AL dams exhibited circadian oscillations with a peak at Zeitgeber time (ZT) 12 and a trough at ZT0. MG weight was higher ($P < 0.01$) in VN dams for the first 18 h on 4X, but then decreased. Placement of VN dams back onto AL nursing during d 2 of the study decreased ($P < 0.01$) MG weight during the subsequent 18 h. On d 1, both Bmal and Per2 mRNA levels exhibited circadian oscillations with peaks at ZT0 and 18, respectively, and troughs at ZT12 and 6, respectively. In AL dams, both Lalba and B4galt1 mRNAs also oscillated, with troughs at ZT12 and peaks at ZT18. Both Per2 and B4galt1 were affected ($P < 0.05$) by decreased nursing frequency in the VN dams on d 1. For Per2, the response was an increase in amplitude at ZT18 ($P < 0.02$) while B4galt1′s expression pattern was decreased ($P < 0.0001$). These differences resolved during d 2 when VN dams were placed on AL nursing. These results support the conclusion that decreased nursing frequency alters circadian gene expression and could influence lactose synthesis through dampening the expression of B4galt1. This project supported by National Research Initiative Competitive Grant no. 2007–35206–17831 from the USDA Cooperative State Research, Education, and Extension Service and by the USDA/ARS Cooperative Agreement #6250–51000–048.

Key Words: lactation, milking frequency, circadian

M134 Functional analysis of swine mammary gland transcriptome during late gestation using two bioinformatics approaches. W. S. Zhao*[1,2], K. Shahzad[1], D. E. Graugnard[1], J. Luo[2], J. J. Loor[1], and W. L. Hurley[1], *[1]University of Illinois, Urbana, [2]Northwest A & F University, YangLing, Shaanxi, China.*

As in other mammals, sow mammary glands are essential to support growth and development of neonatal piglets via the synthesis of milk. We used a newly developed dynamic impact approach (DIA) and the online bioinformatics tool DAVID to perform functional analysis of the sow mammary transcriptome during pregnancy. Mammary tissue was harvested at slaughter on d 80, 100 and 110 of gestation. A swine oligoarray (70 mer) with 13,263 inserts was used for transcriptome profiling. An ANOVA with false discovery rate (FDR < 0.15) correction resulted in 1,539 genes with a significant time effect. Bioinformatics analysis using DIA included the entire data set with Entrez gene ID, FDR, fold-change, and post-hoc *P*-values between time points ($P < 0.05$; d 110 vs. 80, d 110 vs. 100, d 100 vs. 80). For the DAVID analysis, a cut-off of FDR < 0.15 and *P*-value <0.05 was used. By implementing a criterion of mean impact +1 SD to the final results, a total of 14 highly impacted KEGG pathways were uncovered during gestation. Within the ‘Metabolism’ KEGG category, DIA uncovered that ‘one carbon pool by folate’, ‘galactose metabolism’, and ‘fatty acid biosynthesis’ were among the most-impacted pathways on d 100 and 110 vs. d 80; whereas ‘linoleic acid metabolism’ was inhibited overall on d 100 and 110 vs. d. 80. Within ‘Genetic Information Processing’ in KEGG, protein export was markedly activated at d 110 compared with d 80 and 100. Within ‘Environmental Information Processing’ KEGG category, ‘ABC transporters’ was the most-induced pathway during late gestation, while ‘ECM-receptor interaction’ was inhibited at d 110 vs. 80. The latter category was markedly activated at d 110 vs. 100. Within the ‘Organismal Systems’ KEGG category, ‘intestinal immune network for IgA production’ was markedly induced during d 110 and 100 vs. 80. Similar findings were obtained using DAVID approach. Overall, preliminary results from both bioinformatics approaches indicated that folate-mediated one carbon metabolism may serve an important metabolic role in preparation for copious milk synthesis. In addition, the immune system properties of the mammary gland also seem to play an important role in allowing the organ to recognize potential pathogens. Such response may be a mechanism of the mammary gland to prevent invasion of microbes during late gestation.

Key Words: systems biology, microarray, transcriptomics

M135 Changes in milk composition of Holstein dairy cows within a milking. D. E. Rico,* E. R. Marshall, and K. J. Harvatine, *Penn State University, University Park.*

The variation in milk composition within a milking was studied in high producing cows. Eight multiparous Holstein cows (54.86 ± 6.8 kg milk/d; mean ± SD) fed a 31.5% NDF and 17.0% CP diet were used in the experiment. A milk-sampling device was designed to allow collection of multiple samples during a milking without loss of vacuum or interruption of milk subsampling. The average milk yield of the previous 7 milkings was used to determine 5 equal weight sampling intervals. If milk yield exceed expected by more than 25% of the interval weight a sixth sample was collected. Milk was collected during consecutive morning and afternoon milkings for all cows and was replicated one week later. Each sample representing approximately 20% of the milking was analyzed for fat, true protein, and lactose by infrared spectroscopy. A second subsample was stored at −20°C without preservative for analysis of FA composition. Data were analyzed using the Proc Mixed procedure (SAS institute) as a random regression. The model included the random effect of cow and week and the fixed effect of milking fraction, time of milking (AM vs PM) and the interaction of milking fraction by time of milking. Denominator degrees of freedom were calculated by the Satterthwaite procedure. Milking fraction (MF) was a continuous variable calculated as the midpoint of the sampling interval. There was an effect of milking (AM vs PM) on total milk yield and milk protein, lactose, and fat concentration. There also was an interaction of milking time (AM vs PM) and milking fraction, and a quadratic effect of milking fraction on milk fat, protein and lactose concentration ($P < 0.001$). Milk fat concentration exhibited the most marked change during milking and the best fit predictions for the AM and PM milkings were $1.43 + 1.65 \times MF + 2.71 \times MF^2$ and $1.89 + 1.42 \times MF + 2.7124 \times MF^2$, respectively. Milk fat content increased quadratically over the course of milk let down in high producing dairy cows, while much smaller changes were observed in protein and lactose. This pattern is consistent with previous

results in lower producing dairy cows and reflects the dynamic nature of milk fat secretion from the mammary gland.

M136 Osteopontin secretion in milk is correlated to the presence of DNA polymorphisms in the secreted phosphoprotein 1 (*SPP1*) gene. P.-L. Dudemaine*[2] and N. Bissonnette[1,2], *[1]Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Center, Sherbrooke, QC, Canada, [2]Université de Sherbrooke, Sherbrooke, QC, Canada.*

Osteopontin (OPN), encoded by secreted phosphoprotein 1 (SPP1) gene, is a phosphoglycoprotein that is present in various tissues and secreted into body fluids, such as bovine milk. It is recognized as an important pro-inflammatory cytokine which has numerous functions, including its role in early T-cell activation during bacterial infection. The goal of the study was to evaluate the impact of polymorphisms in the *SPP1* gene on OPN secretion in milk. In a previous study, elevated OPN expression in response to mammary gland infection has been characterized. Furthermore, several polymorphisms detected in the 5′ untranslated region of the gene (promoter) have an impact on the estimated breeding value (EBV) for somatic cell score (SCS), an indicator of udder health. Also, haplotypes were defined for low (H1 × H4) and high (H2 × H3) SCS. In dairy cows, OPN is secreted during lactation and could be involved in the mammary gland immunity. In the present study, the main objective was to determine whether polymorphisms in the *SPP1* gene are related to the presence of isoforms or the level of OPN in milk. An antibody panel was used to characterize OPN isoforms by immunoblotting and to measure the amount of OPN secreted in milk. This enabled the selection of the most suitable antibodies to develop an ELISA. The sensitivity of the ELISA was determined at 220 pg/mL with a recovery of 94 ± 5%. Variations in OPN milk concentrations were detected during lactation for cows representing haplotype H1 × H4 (n = 3) and H2 × H3 (n = 3). Elevated concentration in colostrum (678.5 ± 68.7 mg/L) drastically decreased within the first week to reach 19.3 ± 3.7 mg/L of milk, and then remained constant until d 150. From d 150 to 200, cows with haplotypes associated with a low EBV for SCS had increased OPN level in milk ($P < 0.0006$) compared with the cows with haplotype associated with high EBV. This is the first study to demonstrate that a cow′s *SPP1* genetics affects OPN concentration in milk.

Key Words: osteopontin, bovine lactation, genetic variation

M137 Growth hormone influences mTORC1 and IGF-1 signaling in the lactating bovine mammary gland. S. McCoard,* Q. Sciascia, and D. Pacheco, *Animal Nutrition Team, AgResearch Grasslands Limited, Palmerston North, New Zealand.*

The objective of this study was to determine whether mTOR and IGF-1 signaling mediates the effect of growth hormone (GH) on milk protein synthesis in the bovine mammary gland. Mammary tissue was obtained post-mortem from non-pregnant second parity Jersey cows (178–200 d postpartum) 6 d after a single subcutaneous injection of either a slow-release formulation of commercially available GH (Lactatropin) 500mg; (n = 4) or saline (n = 4). Total DNA, RNA and protein were extracted using TRIzol reagent and relative ratios used to evaluate cell size, number and translational efficiency and capacity. The abundance and phosphorylation status of targets of mechanistic target of rapamycin complex 1 (mTORC1) and insulin-like growth factor-1 (IGF-1) signaling were evaluated using Western blotting. Expression of IGF-1 signaling targets was evaluated using qPCR and the data analyzed using LinRegPCR and REST software. Treatment with GH increased mammary gland weight (33%; $P = 0.03$) and total protein content (34%; $P = 0.05$) and tended ($P < 0.10$) to increase ribosome number (33%) and cell size (30%) and DNA concentration (20%), while protein synthetic efficiency, capacity and cell number were unchanged ($P > 0.10$). These results suggest that GH stimulation of global protein synthesis is mediated by an increase in ribosome number and cell size. Abundance of mTOR and mTOR-Ser2448 (1.5 fold, $P < 0.05$), eIF4E (1.8 fold, $P < 0.05$), eIF4E-Ser209 (2.6 fold, $P < 0.01$), the eIF4E-eIF4G complex (1.8 fold, $P < 0.01$) and eIF4E-4EBP1 complex (1.3 fold, $P < 0.05$), MKNK1 (1.5 fold, $P < 0.05$), phosphor-MKNK1 (2.1 fold, $P < 0.01$), RPS6KA1 (1.7, $P < 0.01$) were increased in response to GH, but 4EBP1, p70S6K1 and p85S6K1 were unchanged. Expression of IGF1BP3 (1.4 fold, $P < 0.01$) and IGF1BP5 (1.6 fold, $P < 0.01$) were increased, IGF-1 receptor (1.3 fold, $P < 0.01$) was decreased, and IGF-1 was unchanged in response to GH. Overall, these results indicate that the mTOR pathway may mediate the effect of GH on ribosome biogenesis and cell size, while GH-activation of global protein synthesis is linked to changes in the abundance, phosphorylation and association state of components of the IGF-1 signaling pathway and downstream targets of translational regulation.

Key Words: mTOR pathway, bovine, mammary gland

M138 First demonstration of decorin, an extracellular matrix molecule, in bovine mammary tissue. K. M. O'Diam*[1], S. G. Velleman[1], V. A. Swank[1], S. Ellis[2], A. V. Capuco[3], and K. M. Daniels[1], *[1]Department of Animal Sciences, The Ohio State University, OARDC, Wooster, [2]Animal and Veterinary Sciences Department, Clemson University, Clemson, SC, [3]Bovine Functional Genomics Lab, USDA-ARS, Beltsville, MD.*

In the mammary gland, an extracellular matrix (ECM) is secreted by and surrounds cells located in both mammary parenchyma (PAR) and stroma. Decorin is an ECM proteoglycan with cell growth regulatory effects mediated by its ability to interact with growth factors or upregulation of cyclin-dependent kinases. It also serves a structural role by regulation of collagen crosslinking. Based on these known roles, we undertook a pilot scale study to examine the spatial and temporal localization of decorin in bovine mammary tissue at various physiological stages. Mammary PAR (n = 6) was obtained from female Holsteins (n = 4). Samples represented 3 prepubertal times (d40, 56 and 100 of postnatal life), 2 gestational times (d213 and 248 of gestation) and one lactating time point (d10 post calving). Slides were subjected to immunohistochemical staining for the decorin antigen. A horseradish peroxidase conjugated secondary antibody and diaminobenzidine chromagen were used for detection. The negative control for the assay was incubated with control sera instead of the decorin primary antibody; all other procedures were the same. The negative control was free of chromagen. Our analyses demonstrated that decorin is present in bovine mammary ECM. Furthermore, temporal and spatial localization changed with physiological stage. At all time points observed, except for d56, decorin was not observed immediately surrounding epithelial cells comprising ducts. Across all time points, decorin localization in the intralobular stroma was minimal. Interlobular stroma showed a temporal distribution pattern of decorin staining. At d40, the gestational time points and d10 post calving, decorin localization was intense; at the d56 and d100 prepubertal time points, comparatively less decorin was localized in the interlobular stroma. To the best of our knowledge, this report is the first demonstration of the localization of decorin in bovine mammary PAR. Given our observation that localization appears to vary with physiological state, decorin may be important in the regulation of growth, development and remodeling of bovine mammary gland.

Key Words: decorin, extracellular matrix, mammary gland

M139 MicroRNA expression patterns are affected by stage of lactation in dairy cattle mammary gland. M. Z. Wang*[1,2], S. Moisa[2], D. Bu[1], J. Wang[1], and J. J. Loor[2], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]University of Illinois, Urbana.*

MicroRNA (miRNA) are small non-coding RNA which regulate gene expression post-transcriptionally and play a key role in development and specific biological processes. The human breast-specific signature is characterized by the expression of 23 miRNA and the mouse mammary-specific signature is characterized by the expression of 9 miRNA (let-7a, let-7b, let-7c, miR-26a, miR-26b, miR-24–2, miR-145, miR-30b, and miR-30d). Those data suggest that miRNA could play a role in mammary gland physiology. Knowledge of the expression patterns of miRNA in bovine mammary gland during the transition from pregnancy into lactation might provide insights into their role in regulating aspects of metabolism, differentiation and apoptosis, and immune response. The objective of this work was to determine the expression pattern of selected miRNA involved in cellular proliferation, lipid metabolism, and innate immunity in dairy cow mammary gland tissue at different stages of lactation. Cows from the University of Illinois Dairy Research farm were used in this study. The expression of miR-10a, −15b, −16, −21, −31, −33b, −145, −146b, −155, −181a, −205, −221, and −223 was studied by RT-PCR in tissue (n = 7/stage) harvested via repeated biopsy during the dry period (−30 d prepartum), early lactation (7 d postpartum), and peak lactation (30 d postpartum). The results showed that expression of all miRNA, except miR-31, increased markedly between the dry period and early lactation. Among those, the expression of miR-221 increased further by peak lactation potentially suggesting a role in the control of endothelial cell proliferation/angiogenesis; whereas, the expression of miR-223 decreased by peak lactation but to a level that was greater than the dry period suggesting it may play a role in the mammary response to pathogens soon after parturition. The expression of miR-31, a hormonally regulated miRNA that inhibits cyclin gene expression, was greater at peak lactation vs. the dry period. From a metabolic standpoint, the consistent upregulation of miR-33b during early lactation vs. dry period suggests that this miRNA may exert control over lipogenesis in mammary tissue as it does in rodents. Collectively, results indicated that expression of miRNA associated with regulation of transcription of genes across diverse biological functions is altered by stage of lactation. Determining the specific roles of these miRNA during lactation requires further research, including examination of the expression patterns of their target genes.

Key Words: metabolism, genomics, bioinformatics

M140 Proteomic analysis in MAC-T cells reveals proteins involved in *cis*-9, *trans*-11 CLA de novo synthesis. T. Wang,* J. J. Oh, D. C. Piao, J. H. Hwang, Y. C. Jin, S. B. Lee, K. H. Lee, J. N. Lim, H. S. Kang, and H. G. Lee, *Department of Animal Science, Pusan National University, Miryang, Gyeongnam, Korea.*

This study was conducted to explore proteins involved in *cis*-9, *trans*-11 CLA de novo synthesis through proteomic analysis in MAC-T cells. Cells were routinely cultured in DMEM growth medium at 37°C and 5% CO_2. After reaching 100% confluence, the cells underwent differentiation in lactogenic medium for 90 h. Treatments were performed in triplicate with BSA (control) or t11 C18:1 (50 μM in BSA) and samples were collected every half hour for 4 h. Lipids were extracted following the Folch method and the lipid methyl esters were quantified by GC with a SP-2560 fused silica capillary column. The expression of SCD1 protein was detected by Western blotting using a specific mouse monoclonal primary antibody to SCD. On the basis of the SCD1 expression in 2.5 h, the difference of protein expression among control and t11 C18:1 group were compared by 2-DE. The striking differently expressed spots (≥2 or ≤ 0.5) were identified using ESI-Q-TOF and a protein search engine. Results showed that t11 C18:1 continually converted into *cis*-9, *trans*-11 CLA in MAC-T cells and showed a positive correlation (R^2 = 0.887) during the test. The SCD1 protein expression increased more than 2-fold at 2 h and 2.5 h whereas it decreased by half at 4 h. One upregulated and 6 downregulated proteins were identified in 2.5 h. The expression pattern of the upregulated protein (RASGRP4) (0–3.5 h) was positively related (R^2 = 0.466) to that of SCD1, however 3 of the 6 downregulated proteins (PGAM1, ANXA3, TPMT) were negatively (R^2 = 0.280, R^2 = 0.297, R^2 = 0.182) related to SCD1. Our results suggest that these identified proteins may be involved in the *cis*-9, *trans*-11 CLA de novo synthesis of MAC-T cells.

Key Words: *cis*-9, *trans*-11 CLA de novo synthesis, MAC-T cells, proteomic analysis

M141 Potent growth promoting activity of prolactin and estrogen to E-cadherin/β-catenin adhesion molecules in bovine mammary gland: Modulation of Wnt signaling. J.-J. Tong, Q.-Z. Li,* X.-J. Gao, N. Zhang, and Y. Lin, *Key Lab of Dairy Science, Ministry of Education, Northeast Agriculture University, Harbin, Heilongjiang, China.*

The Wnt signaling pathway and subsequent upregulation of β-catenin driven downstream targets (e.g., cyclinD1) are associated with development of mammary gland. E-cadherin is a key molecule component of the cell-cell adhesion junctions, which plays a principal role in maintaining the morphogenesis of numerous organs including mammary gland. Therefore, the objectives of this presentation is that to fully elucidated the molecular mechanisms underlying the effects of prolactin and estrogen on E-cadherin, β-catenin and cyclinD1 in Wnt signaling pathway in bovine mammary epithelial cells (BMECs). The effect of prolactin and estrogen (5 μg/mL) treatment on expression the key elements of Wnt signaling pathway components in BMECs was analyzed at mRNA level and protein level by qRT-PCR, immunofluorescence and Western blotting. Our present findings showed that prolactin and estrogen effectively promoted the expression of E-cadherin and cyclin D1 on mRNA level ($P < 0.05$), as well as protein level ($P < 0.05$), tested by by qRT-PCR, immunofluorescence and Western blotting. The analysis of β-catenin protein showed that prolactin and estrogen can markedly reduce the nuclear β-catenin expression at protein level. Furthermore, we suggest that modulation of Wnt signaling may be one of the mechanisms implicated the effect on cell proliferation in BMECs by prolactin and estrogen. We demonstrated that prolactin and estrogen can activate cell proliferation in BMECs tested by Reagent WST-1 ($P < 0.05$). Prolactin and estrogen enhanced Wnt signaling through increasing E-cadherin on protein level by lowering the levels of intracellular β-catenin and the influence on the important component of Wnt pathway target-cyclinD1. In conclusion, we were first focused on the effect of prolactin and estrogen on modulation of key components of Wnt signaling components in BMECs. Our results also highlighted the importance of Wnt components—E-cadherin, β-catenin, cyclinD1 that may serve as future subjects in the study of development and lactation of the mammary gland in dairy cattle.

Key Words: Wnt pathway, E-cadherin, bovine mamary epithelial cells