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366 Identification of a short isoform of the porcine prolactin receptor and its variants. J. F. Trott*, A. Schennink, and R. C. Hovey, *University of California, Davis.*

Prolactin (PRL) acts through the dimerizing PRL receptor (PRLR) to regulate more than 300 biological processes, particularly during reproduction and lactation, which are both critical for successful swine production. The actions of PRL are mediated by both long (LF) and short isoforms (SF) of the PRLR, where SF can interfere with the essential signaling function of the LF. We have cloned a SF of the porcine PRLR using 3' RACE. This novel isoform of the pPRLR contains a short intracellular domain of 38 aa that is encoded by splicing from exon 9 to exon 11 on chromosome 16, where the LF splices from exon 9 to exon 10. Analysis of the expression of pPRLR-LF and short pPRLR-SF mRNA by qRT-PCR revealed differential expression of these isoforms with an average ~1000-fold higher level of expression of pPRLR-LF mRNA in 19 tissues. We have also identified polymorphisms within exon 11 that give rise to 4 different coding sequences for the pPRLR-SF. Full-length cDNA for all 4 alleles was generated by PCR and cloned behind the elongation factor 1- α promoter for functional studies, using transiently-transfected of Chinese Hamster Ovary cells. None of the pPRLR-SF alleles were able to activate 342 bp of rat β -casein promoter in response to insulin, dexamethasone and pPRL. All 4 pPRLR-SF alleles functioned as dominant-negatives against the differentiative function of the long pPRLR, where 2 of them completely inhibited long pPRLR activity when they were present at 4-fold excess ($P < 0.05$) whereas the other 2 required 6-fold higher levels to have the same effect ($P < 0.05$). The binding affinity of the 4 alleles of these pPRLR-SF for pPRL was unaffected by differences in the intracellular domain coding sequence ($P > 0.05$). In conclusion, we have identified a unique pPRLR-SF that suppresses the differentiative function of the pPRLR-LF, where the 4 novel pPRLR-SF sequences may differentially impact the phenotypic effects of PRL in swine.

Key words: prolactin, prolactin receptor, short form

367 Comparative transcriptome analysis of laser microdissected cells from bovine mammary gland. K. M. Daniels*¹, R. K. Choudhary², C. M. Evock-Clover³, R. W. Li³, W. Garrett³, and A. V. Capuco^{3,2}, ¹The Ohio State University, Wooster, ²University of Maryland, College Park, ³USDA-ARS, Beltsville, MD.

Bovine mammary parenchyma (PAR) is a heterogeneous tissue. Laser microdissection (LMD) offers a refined strategy for excision, capture and interrogation of user-defined cell populations. Our objective was to use LMD to obtain homogenous cell populations from prepubertal PAR, followed by microarray analysis to characterize molecular signatures of various cells in PAR and to gain insight into interactions among cell types. Cryosections of PAR from 5 prepubertal Holstein heifers were prepared. Each slide was fixed in acetone:polyethylene glycol and stained with 0.1% nuclear fast red in 5% AlSO_4 , rinsed with PBS and dehydrated through 3 grades of ethanol. Cells from 4 locations within and near terminal ductal units (TDU) were captured. Basal layer and embedded layers of epithelium were obtained, as were intralobular and interlobular stroma. The 4 categories of cells from each heifer were lysed, cDNA synthesized, amplified and labeled for microarray analysis. The microarray (Roche Nimblegen, Inc.) represented over 45,000 bovine sequences and over 3800 genes were included in the analysis. Transcriptome analysis ($P \leq 0.05$; ≥ 2 -fold

change) of basal vs. embedded epithelial cells showed many genes involved in differentiation, with an enrichment of transcripts for nestin (stem cell marker), a noncoding maternally imprinted gene (H19), extracellular matrix proteins and metalloproteinases in the basal epithelium. Conversely, there was enrichment of a telomerase inhibitor (POT1) and phosphodiesterases in the embedded epithelial cells. Evaluation of gene expression in the basal epithelium vs. adjacent intralobular stroma showed greater expression of keratin 7, Msh homeobox (MSX2; likely morphogen) and protein tyrosine phosphatase 14. There was an enrichment of MEOX2, a mesenchyme inducer, and sphingosine-1-phosphate receptor 1. Results suggest presence of stem or progenitor cells in the basal epithelium and induction of myoepithelial differentiation via stromal factors. Comparisons of interlobular and intralobular stroma showed a prevalence of lipid associated genes (SCD, DGAT2) in the former. Utility of LMD in bovine mammary tissue was demonstrated.

Key words: laser microdissection, microarray

368 Acute DNA methylation changes are associated with involution and re-initiation of lactation in dairy cows. K. M. Swanson*¹, K. Stelwagen², R. A. Erdman³, and K. Singh¹, ¹AgResearch Ltd., Ruakura Research Centre, Hamilton, New Zealand, ²Agri-Search, Hamilton, New Zealand, ³University of Maryland, College Park.

The onset of bovine mammary gland involution following the termination of milking results in a decline in milk protein gene expression, including the major milk protein α S1-casein. In a previous study DNA methylation, in the α S1-casein-encoding gene at a functional STAT5 binding site approximately -10kbp, was increased after 7d of involution. The aim of the present study was to determine whether DNA methylation plays an acute role in the downregulation of milk production during the reversible and irreversible phases of involution. Non-pregnant cows at mid-lactation were divided into 5 groups ($n = 5$ /group). Mammary alveolar tissue was obtained at slaughter from lactating cows 6 h post-milking (control), cows with extended non-milking periods of either 7- or 28-d, and cows where milking was resumed for 7 d following these 2 dry periods. Re-initiation of milking following 7- or 28-d non-milking periods resulted in milk yield recoveries of 93 and 25% respectively. Quantitative MassARRAY methylation analysis revealed that methylation levels of 2 5methylCpG dinucleotides at the STAT5-binding site -10kb in the α S1-casein promoter were increased to 32% ($P < 0.1$) and 33% ($P < 0.05$) respectively following 7-d non-milking, then further to 38% ($P < 0.05$) and 50% ($P < 0.05$) respectively following 28-d non-milking, compared with lactating cows (22% and 13% methylation, respectively). Furthermore, the full recovery of lactation following re-initiation of milking after 7-d non-milking was associated with de-methylation of DNA, returning to levels similar to lactating control cows. However, the DNA methylation levels remained significantly higher ($P < 0.05$) than the lactating control cows following the re-initiation of milking after 28-d non-milking, in association with the low, partial recovery of milk yields. These results suggest DNA methylation at a functional STAT5-binding site of the α S1-casein-encoding gene may play a role in regulating reversible and irreversible involution.

Key words: DNA methylation, bovine α S1-casein, mammary involution

369 Ontogeny of nuclear and cytoplasmic myoepithelial markers during prepubertal bovine mammary development. S. Safayi*¹, N. Korn¹, A. Bertram¹, R. M. Akers², A. V. Capuco³, S. L. Pratt¹, S. Calcaterra¹, C. Klein¹, and S. Ellis¹, ¹Clemson University, Clemson, SC, ²Virginia Polytechnic Institute and State University, Blacksburg, ³USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD.

We previously reported that ovariectomy alters prepubertal mammary myoepithelial cell (MC) development, but the mechanisms involved are not well understood. We therefore surveyed expression of transformation-related protein 63 (P63) and the common acute lymphoblastic leukemia antigen (CD10) as differentiation markers to track the ontogeny of MC development. At 40d of age, Holstein heifers underwent either an ovariectomy (OVX; n = 16) or sham (INT; n = 21) operation. At d55, 70, 85, 100, 130 and 160 of age, groups of heifers were slaughtered to provide mammary parenchyma samples for multispectral imaging and subjective assessment of immunofluorescent staining for MC markers. Our qualitative evaluation showed P63-/CD10+, P63+/CD10-, double positive and double negative cells in both basal and suprabasal layers. The P63+ nuclei were observed in the basal layer at regularly spaced intervals. The interval between P63+ nuclei was reduced in older heifers regardless of treatment. Stromal CD10 expression also appeared to be more prominent in older heifers. In both OVX and INT heifers, P63 and CD10 expression was more heterogeneous at the distal ends of the ductular units compared with the subtending ducts. In INT heifers, there was a reduction in the interval between P63+ MC nuclei, compared with OVX heifers. Our observations suggest that expression of P63 in the basal layer of parenchyma from INT heifers was more consistent than in OVX animals. Our results provide support for the hypothesis that ovarian secretions affect the expression of both P63 and CD10. Additional quantitative analyses are now required to substantiate our assertions from the qualitative inspection.

Key words: myoepithelial cell, p63, CD10

370 Multispectral analysis of myoepithelial cell development in prepubertal bovine mammary gland. S. Safayi*¹, N. Korn¹, A. Bertram¹, R. M. Akers², A. V. Capuco³, S. L. Pratt¹, and S. Ellis¹, ¹Clemson University, Clemson, SC, ²Virginia Polytechnic Institute and State University, Blacksburg, ³USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD.

We have previously reported that ovariectomy alters prepubertal development of mammary myoepithelial cells (MC), but the mechanisms involved are not well understood. We therefore analyzed the MC expression of differentiation markers α -smooth muscle actin (SMA) and the common acute lymphoblastic leukemia antigen (CD10). On d40, Holstein heifers underwent either an ovariectomy (OVX; n = 16) or sham (INT; n = 21) operation. At d55, 70, 85, 100, 130 and 160 of age, samples were collected and used for multispectral imaging to quantitatively assess immunofluorescent staining for target MC markers. Fluorescent intensity (FI) of the markers on each slide were normalized against a control sample and then evaluated statistically using PROC MIXED in SAS. Our results showed 250% more CD10+ cells contacting the luminal space in OVX compared with INT ($P = 0.04$). In the basal layer, CD10 FI was lower ($P = 0.04$) and SMA FI was higher ($P < 0.01$) in OVX than INT. The FI ratio of SMA to CD10, as a proxy indicator for MC differentiation, increased in OVX compared with INT after d55 ($P < 0.01$), and remained approximately 4 times higher from d100 onwards ($P < 0.01$). Intracellular distribution of markers within the basal layer showed basal localization of SMA, but apical CD10 staining. In both INT and OVX, there were also double nega-

tive cells expressing neither CD10 nor SMA that appeared to contact both the basal and luminal spaces. Our results show that ovariectomy affects MC expression of both SMA and CD10 as well the pattern of MC development. Given that MC are known to limit parenchymal growth in other species; our observations open new avenues for future studies on the regulation of prepubertal mammary development.

Key words: myoepithelial cell, CD10, smooth muscle actin

371 Lactogenic hormones and IGF-I do not regulate glucose transporter gene expression in the bovine mammary gland during the transition period. Y. Shao*¹, E. Wall¹, Y. Misra¹, X. Qian¹, R. Blauwiel¹, T. McFadden², and F.-Q. Zhao¹, ¹Laboratory of Lactation Physiology, Department of Animal Science, University of Vermont, Burlington, ²Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.

Glucose is essential for milk production as it is a major substrate and energy source for milk synthesis. Glucose uptake across the plasma membrane of mammary epithelial cells is mediated by transmembrane facilitative glucose transporters (GLUT). During the transition period, there is a marked increase in the expression of GLUT1, GLUT8, and GLUT12 in the bovine mammary gland. The objective of this study was to investigate whether the lactogenic hormones and/or IGF-I are the mediators of increased GLUT expression. In the first experiment, mammary tissue was obtained by biopsy from 2 primi- and 2 multiparous cows about 40 d before parturition. Mammary explants were cultured for 48, 72 and 96 h with the following hormone treatments: no hormone (control); 200 ng/ml IGF-I; 5 μ g/ml insulin (INS); 5 μ g/ml insulin + 1 μ g/ml hydrocortisone + 5 μ g/ml prolactin (IHPrI); and 5 μ g/ml insulin + 1 μ g/ml hydrocortisone + 5 μ g/ml prolactin + 500 ng/ml estrogen (IHEPrI). The relative expression of β -casein, α -lactalbumin, GLUT1, GLUT8 and GLUT12 mRNA were measured by real time PCR. For β -casein and α -lactalbumin, IGF-I and INS had no effect, whereas IHPrI and IHEPrI increased mRNA several hundred fold ($P < 0.01$). Although insulin alone increased GLUT1 mRNA around 1.8 fold ($P < 0.05$), IGF-I, IHPrI and IHEPrI had no effect on GLUT1 or GLUT8 expression. There was no treatment effect on GLUT12 expression at 48 h, but IHPrI and IHEPrI decreased GLUT12 expression by 50% after 72 and 96 h ($P < 0.05$). In the second experiment, prolactin was administered twice a day to 5 cows during early lactation by intravenous injection at a dose of 1 μ g/kg of BW. After 7 d of treatment, mammary tissue was obtained by biopsy from prolactin-treated cows and 5 control cows. Expression of GLUT1, 8 and 12 mRNA was measured by real time PCR, and there was no effect of prolactin on gene expression. Our data suggest that lactogenic hormones and IGF-I may not mediate the increase in GLUT expression in bovine mammary gland during the transition period.

Key words: glucose transporters, bovine mammary gland, lactogenic hormones

372 Lactogenic complex-induced mammary epithelial cell differentiation is associated with membrane compositional differences. N. Argov-Argaman*, K. Mida, and A. Shamay, *The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University, Jerusalem, Israel.*

Mammary gland epithelial cell differentiation is induced by insulin (I), hydrocortisone (F) and prolactin (PrI). Although the mammary epithelial cell proteome is well established, differentiation-associated changes in its lipidome composition are not well defined. A murine mammary

gland epithelial cell line (HC11) was used to study alterations in lipid composition of the cell and its membrane during differentiation. HC11 cells were exposed to one of the following hormonal treatments: IPrl, PrIF, and IF. Differentiation medium consisted of prolactin, insulin and hydrocortisone ($n = 3$ for lipid and 4 for gene expression analysis; 2 biological replicates for each experiment). Lipid composition of fat and membrane cellular compartments were determined. Gene-expression levels of lipogenic and lipolytic enzymes, as well as of genes encoding enzymes that modulate fatty acid length and unsaturation level (i.e., elongases and desaturases, respectively) were measured. Overall, insulin was the main factor inducing alterations in membrane composition, which consisted of increased phosphatidylethanolamine ($12+0.5$ and $15+0.4$ for PrIF compared with IFPrI, $P = 0.004$) and decreased sphingomyelin concentrations ($2+0.3$ and $37+0.4$ for PrIF compared with IFPrI, $P = 0.0002$). Nonetheless, the differentiation medium, (IFPrI), had a stronger effect on phospholipid composition than all other treatments which included insulin. In addition, insulin increased the concentration of monounsaturated fatty acids ($46+0.5$ and $53+0.1$ for PrIF compared with IFPrI, $P < 0.001$), but decreased that of polyunsaturated fatty acids in the membrane ($17.5+0.3$ and $11.1+0.1$ for PrIF compared with IFPrI, $P < 0.001$). Gene expression of both desaturases and elongases (ELOVL 1, 3, 5, 6 and 7, $\Delta 5$, $\Delta 6$ and $\Delta 9$ desaturase) was elevated by differentiation medium only relative to all other treatments. The results suggest that membrane compositional differences in both polar lipids and fatty acids occur concomitantly with the differentiation of the mammary epithelial cells and suggest a role for membrane lipid composition in the acquisition of mammary cell characteristics.

Key words: milk, membrane, phospholipid

373 Intravenous supplementation of acetate, glucose or essential amino acids to an energy and protein deficient diet in lactating dairy goats: effects on milk production and mammary nutrient extraction. S. Safayi^{1,2} and M. O. Nielsen¹, ¹University of Copenhagen, Frederiksberg, Great Copenhagen, Denmark, ²Clemson University, Clemson, SC.

The objectives were to study how mammary supply of essential amino acids (EAA) versus energy yielding substrates in the form of acetate (ACE) or glucose (GLU) would affect mammary nutrient uptake and milk (protein) synthesis in early (EL) and late lactating (LL) dairy goats. Four goats were fed a basal diet deficient in energy (90% of requirements) and protein (80% of requirements), and randomly allocated to 4 treatments in a balanced 4×4 Latin Square design. The treatments consisted of 4-d continuous intravenous infusions of isoosmotic isoenergetic solutions of EAA, ACE and GLU with saline (SAL) as control, having a 3-d rest period in between. Simultaneous arterio-venous blood samplings over each udder half/gland were performed every 4 h during the last 24 h of infusion. Milk production was recorded, and its fat and protein contents as well as some of the blood/plasma parameters/components were determined. In EL, milk yield or energy corrected milk yield (ECM) was stimulated by GLU ($P = 0.01$) and tended to be increased by EAA ($P = 0.06$) and ACE ($P = 0.06$). ACE and EAA (but not GLU) stimulated milk protein yield

($P = 0.06$ and 0.03 , respectively for ACE and EAA), illustrating the importance of amino acid as well as ATP generation for support of protein synthesis in EL. Highest fat yield in EL was observed on ACE ($P < 0.05$). In LL, only EAA could raise milk protein yield ($P < 0.01$) and ECM ($P = 0.06$). In LL, GLU lowered milk fat percentage ($P = 0.01$). We conclude that an insufficient amino acid supply to the mammary gland of dairy goats can be compensated in EL but perhaps not in LL by increased mammary supply and uptake of an energy yielding substrate, provided this substrate specifically contributes to ATP generation in mammary epithelial cells. This suggests there might be scope for development of differential protein recommendations for ruminants in early and late lactation, and this issue should be pursued in future studies.

Key words: mammary metabolism, mammary nutrient uptake, arterio-venous concentration difference

374 Expression profiles of microRNAs from non- and lactating bovine mammary glands. Z. Li^{1,2}, H. Y. Liu^{1,2}, and J. X. Liu^{1,2}, ¹Institute of Dairy Science, College of Animal Sciences, Hangzhou, P.R. China, ²MOE Key Laboratory of Molecular Animal Nutrition, Hangzhou, P.R. China.

The microRNAs (miRNA) are small non-coding RNA molecules that are approximately 22 nucleotides (nt) in length. It has been reported that miRNAs are involved in the regulation of milk protein synthesis and development of mammary gland. However, little information is available on the function of miRNA in regulation of lactation. Therefore, clarifying of miRNA expression profiles in mammary gland are crucial to the understanding of the mechanism of lactation initiation. In this study, one healthy cow each from both non- and lactating stages was selected to collect mammary gland tissues. Two miRNA libraries were constructed and sequenced, respectively. The miRNAs (18–30nt) were sequenced by Solexa sequencing method. A total of 11,964,909 clean reads were obtained from lactating cows and 15,968,116 clean reads from nonlactating cattle. The sequencing data were analyzed using ACGT101-miR program. There were 962 pre-miRNAs encoding for 985 miRNAs, of which 946 were unique and 521 (52.9%) were expressed in both periods. Among the unique sequences, 284 were known as bovine miRNAs registered in the miRbase database; 96 miRNAs were conserved in other mammalian; and 566 miRNAs were bovine-novel candidates. Real-time quantitative PCR was used for validation of the sequencing results of 32 selected miRNAs which indicated great difference between 2 periods. It is observed that the results of over 50% of these miRNAs were consistent with Solexa sequencing data. There were 17 miRNAs expressed in higher level in lactating than in nonlactating bovine mammary glands. The high percentage of bovine-novel candidates indicated that many miRNAs in bovine mammary gland have not been identified. Further research is warranted to examine what kinds of miRNAs are involved in lactation and how they function in regulation of lactation.

Key words: bovine mammary gland, lactation, miRNAs