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467 The effect of milk accumulation on gene expression in bovine mammary gland. E. H. Wall*[1], J. P. Bond[2], and T. B. McFadden[1], [1]*Department of Animal Science, University of Vermont*, *Burlington,* [2]*Vermont Genetics Network Bioinformatics Core, University of Vermont*, *Burlington*.

We hypothesized that accumulation of milk would influence gene expression in the mammary gland of lactating dairy cows. To test this hypothesis, we enrolled 4 multiparous Holstein cows (150 ± 10 DIM) in a half-udder milk stasis experiment. On d 1 of the experiment, right udder halves were milked at 0430 h and 1430 h. On d 2 of the experiment, right udder halves were milked at 0430 h and mammary biopsies were obtained from both udder halves immediately thereafter. At the time of biopsy, it had been 24 h since left udder halves had last been milked. Using Affymetrix GeneChip Bovine Genome Arrays, we identified 32 genes that were differentially expressed between left (full) and right (empty) udder halves (fold change >1.5; $P < 0.05$). Four of the genes were downregulated in response to milk stasis, whereas 28 were upregulated. Differentially expressed genes were associated with extracellular matrix remodeling, tight junction formation, regulation of blood flow, and apoptosis. In addition, 4 of the differentially expressed genes had been previously identified as candidates for local regulation of milk production in dairy cows. Expression of 2 of these candidates, early growth response-1 (EGR-1) and thrombospondin-1 (THBS-1), was validated using real-time quantitative RT-PCR. Consistent with microarray results, both genes were upregulated in response to 24-h of milk stasis ($P < 0.03$). Immunofluorescence was used to localize expression of EGR-1 protein, which was restricted to epithelia and was uniformly distributed. We conclude that accumulation of milk alters gene expression in the bovine mammary gland. In particular, EGR-1 and THBS-1 have emerged as strong candidates for local regulation of milk production in dairy cows.

Key Words: gene expression, mammary gland, milk stasis

468 Expression of ER stress pathways genes in bovine mammary tissue during the lactation cycle. G. Invernizzi*[1,2], M. Bionaz[1], G. Savoini[2], and J. Loor[1], [1]*University of Illinois*, *Urbana-Champaign,* [2]*University of Milan*, *Milan, Italy*.

Endoplasmic reticulum (ER) has a crucial role in cellular metabolism. Recent studies uncovered a tight relationship between ER stress pathways and lipogenic transcription factors. Mammary gland is subject to extreme metabolic loads at the onset of lactation. Recently, it was discovered that X-box binding protein 1 (XBP1) and eukaryotic translation initiation factor 2-α kinase 3 (PERK) play critical roles in regulating the expression of lipogenic transcription factors such as PPARγ and SREBF1. Furthermore, evidence from non-ruminant cell systems has shown that $p58^{IPK}$ ((DnaJ (Hsp40) homolog, subfamily C, member 3 (DNAJC3)) interacts with PERK to inhibit its eIF2α kinase activity. The latter is induced during the unfolded protein response (UPR) by an ER stress response element in its promoter region. Quantitative real-time RT-PCR of $p58^{IPK}$, PERK and XBP1 in mammary biopsy tissue (n = 5 at each time) was performed at −15, 1, 15, 60 and 240 d relative to parturition. Expression of $p58^{IPK}$ showed peaks ($P < 0.05$) at d 15 and 240 after calving with the highest expression at d 240 (2-fold vs. −15 d). Expression of PERK was similar to $p58^{IPK}$ at d 15 and was significantly increased (1.5-fold) at d 240. A possible unfolded protein response accompanying the sharp increase in milk production after calving (d 15) as well as apoptosis at late lactation (240 d) can partly explain the responses of PERK and $p58^{IPK}$. Preliminary results suggest that the mammary gland experiences ER stress at different stages of the lactation cycle. Further studies could explain better the role of XBP1 in regulation of the ER stress pathways through splicing mechanisms rather than mRNA expression.

Key Words: ER stress, lactation cycle, bovine mammary tissue

469 Effect of dexamethasone and age at induction on milk yields of heifers induced into lactation. A. L. Magliaro-Macrina*[1], A. C. W. Kauf[1], D. A. Pape-Zambito[1], and R. S. Kensinger[2], [1]*The Pennsylvania State University*, *University Park*, [2]*Oklahoma State University*, *Stillwater*.

The objectives of the present study were to determine if age or dexamethasone administration on d 1 and 2 of milking would affect milk production in heifers induced into lactation using estradiol and progesterone. Nonpregnant Holstein heifers at 14 (n = 20; 354 ± 38 kg BW) and 18 mo of age (n = 20; 456 ± 30 kg BW) were randomly assigned to dexamethasone (DEX) or control (CON) treatment groups in a 2 × 2 factorial arrangement with age and DEX as the 2 factors. Heifers were induced into lactation with daily subcutaneous injections of estradiol–17B and progesterone (75 and 250 μg/kg BW/d, respectively) on treatment d 1 to 7. They also received bST every 14 d beginning on treatment d 1. Milking began on treatment d 18 (= d 1 of lactation). DEX (10 mg) was administered on d 1 and 2 following the a.m. milking. CON heifers did not receive DEX. Milk yield from d 2 to 15 of lactation of heifers receiving DEX (7.8 kg/d) was greater ($P < 0.05$), than that of CON heifers (6.0 kg/d) but was similar thereafter until 200 DIM (17.9 kg/d). Milk production to d 11 was similar for 14 and 18 mo old heifers, but milk yield was greater for 18 (19.1 kg/d) than for 14 mo animals (16.7 kg/d) through 200 DIM ($P < 0.01$). Milk fat percent was greater over d 1 to 21 of lactation in DEX (4.48) vs. CON heifers (3.49, $P < 0.01$); milk lactose percent was higher ($P < 0.05$) in DEX (4.40) than CON (4.15) through d 21 of lactation. Day 1 to 7 mean IgG concentration and mass were greater ($P < 0.05$) for 18 (48.1 mg/ml; 41.8 g mass) vs. 14 mo (32.3 mg/ml; 30.0 g mass) old heifers. DEX treatment did not affect IgG content. There were no DEX × age interactions. Administration of DEX to heifers induced into lactation increased d 2 to 15 milk production compared with heifers that did not receive DEX, but not after 15 DIM. DEX appeared to stimulate mammary cell differentiation but did not change the rate of decline of milk IgG concentrations. Higher milk yield and IgG content in 18-mo-old heifers might be due to greater mammary epithelium and/or increased body mass.

Key Words: induced lactation, dexamethasone, dairy heifers

470 Effect of intramammary infusions of fluoxetine (FLX) and 5-hydroxytryptophan (5-HTP) on milk secretion rate and composition in lactating Holstein cows at dry-off. R. J. Collier*1,3, J. L. Collier[1], L. L. Hernandez[2], and N. D. Horseman2,3, [1]*University of Arizona*, *Tucson,* [2]*University of Cincinnati*, *Cincinnati, OH,* [3]*Amelgo*, *Covington, KY*.

Serotonin (5-HT), produced in mammary epithelial cells negatively feeds back on milk secretion via 5-HT receptors in mammary tissue. We hypothesized that increasing 5-HT concentration in milk via inhibiting its reuptake, (FLX) or by increasing the precursor for 5-HT synthesis, 5-HTP would accelerate milk yield at dry-off. Multiparous Holstein cows (45) milked 3x daily and producing at least 20 kg/d were randomly

assigned to once a day milking and one of 3 intramammary treatments (15 cows each) for 3 d. Each infusion was followed by prophylactic antibiotic (Today) on d1 and 2 and Quartermaster and Orbeseal on d 3. The control group (C) received the carrier (sterile H20 and oil at 9:1) The FLX group received 5 mg of FLX and carrier. The 5-HTP group received 5 mg of 5-HTP and carrier. Milk yield and composition samples were obtained daily. Blood samples were obtained by tail venipuncture on d 2 to 4. Rate of milk yield decline was greater for FLX and 5-HTP groups (9.5 and 10.0 kg) compared with C (7.5 kg) on d-1 following initiation of treatments ($P < 0.01$) and did not differ after. Milk lactose, protein and fat % were unaffected by treatment but milk Na:K ratio was increased to 2.8 in FLX treated animals d-2 post-infusion compared with 1.44 in C and 1.78 in 5-HTP treated animals ($P < 0.01$). Plasma lactose was increased 2-fold in FLX treated animals on d-1 post-infusion compared with C and 5-HTP animals ($P < 0.001$). Udder surface temperatures declined as milk yield declined in all animals ($P < 0.001$) and was lower in FLX treated animals than C and 5-HTP treated animals ($P < 0.003$). Rectal temperatures were also reduced in FLX treated animals ($P < 0.03$). We conclude that decrease in milk secretion during the first 24 h after reduction in milking rate from 3x to 1x was accelerated in FLX treated animals. Increase in the milk Na:K ratio and plasma lactose in the FLX groups suggest these treatments altered tight junction function post-dryoff.

Key Words: serotonin, lactation inhibition, dry-off

471 Acute fluoxetine administration accelerates mouse mammary gland involution. L. L. Hernandez*[1], R. J. Collier[2,3], and N. D. Horseman[1,3], [1]*University of Cincinnati*, *Cincinnati, OH,* [2]*University of Arizona*, *Tucson,* [3]*Amelgo*, *Covington, KY*.

Serotonin (5-HT) acts via autocrine-paracrine mechanisms on mammary epithelial cells in a variety of species. In human and bovine mammary epithelial cells, inhibition of the 5-HT reuptake transporter (SERT) with selective 5-HT reuptake inhibitors (SSRIs) exhibit disruption of tight junctions and decreased milk protein mRNA expression. SSRIs act to increase the cellular exposure to 5-HT by preventing reuptake of 5-HT by the cell and eventual degradation. This experiment set out to determine the in vivo effects of fluoxetine (FLX) treatment on lactation in mice. We utilized 7 ICR mice, approximately at 8–10 d of lactation and treated with either a single i.p. injection of sterile saline (CTRL; n = 3) or 40 mg/kg body weight FLX (n = 4). At the time of injection, the number 1, 3, 5 and 7 glands were sealed and the number 2, 4, 6 and 8 glands were left open. Mothers were then returned to their pups for 24 h. After 24 h, lactating dams were sacrificed and the number 1–8 glands were collected from all mothers. Mammary glands were stored in 4% paraformaldehyde overnight, and paraffin embedded and processed for hematoxylin and eosin staining at the Cincinnati Children's Hospital Pathology Research Core. Representative photographs were taken of each gland from each animal and analyzed for alveolar area and number of epithelial cells shed into the alveolar lumen using NIH ImageJ software. The alveolar area and number of epithelial cells shed of 6 alveoli per mammary gland were used for the analysis. Alveolar area was increased in FLX sealed glands relative to CTRL sealed glands ($P = 0.0028$) and in FLX open glands relative to CTRL open glands ($P = 0.04$). The number of epithelial cells shed into the luminal space was increased in FLX sealed glands compared with CTRL sealed glands ($P = 0.0166$) and FLX open glands compared with CTRL open glands ($P = 0.0395$). Results indicate that mammary gland involution was accelerated by systemic FLX treatment in both open and sealed glands compared with CTRL injections.

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Key Words: serotonin, lactation, fluoxetine

472 Effects of early ovariectomy on caprine mammary gland parenchyma during prepuberty. L. Finot[1,2], Y. Yart[1,2], and F. Dessauge*[1,2], [1]*INRA UMR 1080 Dairy Production*, *35590, Saint Gilles, France*, [2]*Agrocampus UMR 1080 Dairy Production*, *35000, Rennes, France*.

In ruminants, ovarian hormones (estradiol and progesterone) are absolutely essential for normal mammary development. From birth to puberty, the mammary parenchyma undergoes rapid growth characterized by cell proliferation and expansion of a ductal network into the surrounding fat pad. The objectives of this study were to increase the understanding of biological mechanisms underlying mammary growth and to investigate the role of ovary secretions during early prepubertal caprine mammogenesis. Alpine young goats were ovariectomized (OVX; n = 9) or sham operated (SHAM; n = 9) at 3 periods before puberty (P1 = 1 mo, P2 = 2 mo and P3 = 3 mo after birth). Goats were harvested at 9 mo of age to remove the mammary gland. Ovariectomy did not influence mammary gland weight at any experimental period. Histological observations revealed that adipose tissue was widely represented compared with secretory tissue (parenchyma) in OVX goats. Morphological analysis of mammary tissues indicated that parenchymal structures of OVX goats were negatively affected by ovariectomy with limited lobules and undeveloped ducts. Ovariectomy at P1 and P2 reduced estrogen receptor α at both the transcriptional (P1 = −85% and P2 = −90%) and translational (P1 = −65% and P2 = −70%) levels. In P1 and P2 periods, ovariectomy strongly affected cell-cell adhesion molecules and extracellular matrix protease activities. Lower expression of E-Cadherin (P1 = −78%; P2 = −76%), Pan-Cadherin (P1 = −60%; P2 = −43%) and P-Cadherin (P1 = −86%; P2 = −75%) was accompanied by a decrease of α and β catenins. In addition, the metalloproteinase MMP2 activity was significantly reduced in ovariectomized animals (P1 = −13% and P2 = −8%). No effects were observed with ovariectomy at 3 mo (P3). In conclusion, ovariectomy at 2 mo of age was the most critical for parenchymal development. These findings suggest that ovary secretions are required to initiate mammary epithelial cell proliferation in prepubertal goats.

Key Words: caprine mammary gland, ovariectomy, estrogen

473 Role of miR–15a in the mammary gland and mammary epithelial cells of dairy cows. H. M. Li, C. M. Wang, and Q. Z. Li*, *Key Laboratory of Dairy Science of Education Ministry, Northeast Agricultural University*, *Harbin, China*.

MicroRNAs (miRNAs) are a class of small non-coding RNAs consisting of 18–25 nucleotides. They regulate the expression of target genes at the post-transcriptional level by degradation or translational inhibition of the complementary mRNA target sequences. The role of miRNA in mammary gland development and lactation is relatively unknown. In this study, qRT-PCR was used to detect the expression of miR-15a and its target gene growth hormone receptor (GHR) in virgin, pregnancy, lactation and involution physiological stages in the mammary gland of Holstein dairy cows, and 3 cows were sampled at each physiological stage. The results revealed that miR-15a and GHR followed the same expression pattern across different physiological states. The expression of miR-15a and GHR was only increased significantly in the sixth month of pregnancy. In the other developmental periods, the expression of miR-15a and GHR was low. To determine the relationship between miR–

15a and GHR, bovine miR-15a was transfected into bovine mammary epithelial cells (BMEC, a cell culture line established by our laboratory). Experiments were replicated 3 times. After miR-15a was overexpressed by transfection, the extent of the miR-15a were 8.4-fold relative to the endogenous miR-15a and the expression of GHR mRNA and protein decreased ($P < 0.01$ and $P > 0.05$, respectively). Flow cytometry showed that over-expression of miR-15a inhibited the proliferation of mammary epithelial cells ($P < 0.01$). In conclusion, these results revealed that miR-15a inhibited the proliferation of mammary epithelial cells as well as the expression of GHR mRNA and protein level. Therefore, this miRNA may play an important role in mammary gland physiology.

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Key Words: miR-15a, GHR, mammary epithelial cell

474 Expression of let–7g in development, lactation and involution of the murine mammary gland. Y. Li, L. Tian, C. M. Wang, and Q. Z. Li*, *Key Laboratory of Dairy Science of Education Ministry, Northeast Agricultural University*, *Harbin, China*.

Micro RNAs (miRNAs) play important roles in the development, lactation and involution of the mammary gland. To identify key miRNAs implicated in mammary gland physiology, healthy female kunming mice in different mammary cycle stages were used (12 time points: virgin 28d, virgin 35d, virgin 49d, pregnancy 2d, pregnancy 9d, pregnancy 13d, lactation 2d, lactation 9d, lactation 13d, involution 2d, involution 5d, involution 10d;and 6 mice were used for each time point). The fourth pairs of abdominal mammary gland tissues were prepared under sterile condition and used for microarray (miRCURY LNA Arrays) and qRT-PCR analysis. Total RNA isolated from mammary gland tissues collected across 12 time points was covalently labeled with Hy3, respectively, and hybridized to the array. The microarray contained 4 replicate subarrays. The data analysis used Genepix Pro 6.0, and GeneSpring 7.2 was used for further data analysis. qRT-PCR was used to confirm the microarray results. Finally, the data were analyzed with SPSS by ANOVA. Expression levels of let-7g changed with the mammary cycle. Microarray and qRT-PCR produced similar results for the expression of let-7g, which exhibited significant changes during the mammary cycle. The microarray showed that let-7g was down-regulated in glands collected during pregnancy compared with virgin and involuting glands ($P < 0.05$). qRT-PCR showed that the expression of let-7g was lower in pregnancy ($P < 0.01$), and was relatively higher in virgin, lactating and involuting glands. To identify genomic targets of let-7g, an algorithm named miRanda and Pic Tar was used. The results showed that let-7g target genes were important transcription factors, such as Myc, Map3k1, Map4k3, and Stat3, which play significant roles in the mammary gland. In conclusion, let-7g was identified as a potential regulator of murine mammary gland development, lactation and involution.

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Key Words: mammary gland, miRNAs, let-7g

475 Effect of heat stress during the dry period on mammary gland development of dairy cattle. S. Tao*, J. W. Bubolz, B. C. do Amaral, M. J. Hayen, S. E. Johnson, and G. E. Dahl, *University of Florida*, *Gainesville*.

Heat stress during the dry period affects immune status, alters hepatic metabolism and decreases milk production in the subsequent lactation. However, cellular mechanisms involved in the mammary response are unclear. Our objective was to evaluate the effects of heat stress during the dry period on mammary gland development of multiparous cows. Cows were dried off 46 d before expected calving and assigned to 2 treatments, heat stress (HT, n = 15) or cooling (CL, n = 14). Average THI during treatment was 76.6 for all cows, but CL cows had sprinklers and fans that came on when ambient temperatures reached 21.1 0C, whereas HT cows were in the same barn without fans or sprinklers. Rectal temperature (RT) was measured twice daily (0730 and 1430 h) and respiration rates (RR) recorded at 1500 h on a Mon-Wed-Fri schedule from dry-off to calving. After parturition, all cows were housed in a free-stall barn with sprinklers and fans. Milk yield was recorded daily to 147 DIM. Mammary biopsies were taken at dry-off, −20, +2 and +20 d relative to calving from a subset of cows (HT, n = 7, CL, n = 7) and infiltrated with paraffin. Numbers of Ki67 immunopositive epithelial and stromal cells were measured in 4-μm mammary tissue sections from each animal. Total cell numbers were measured following hematoxylin histology and percent proliferation was calculated as Ki67+/ total × 100. Compared with HT cows, CL cows had lower morning and afternoon RT (38.6 vs. 38.8 0C, 39.0 vs. 39.40C, $P < 0.01$, respectively) and lower RR (46 vs. 78 breaths/min, $P < 0.01$). Relative to HT cows, CL cows produced more milk (36.5 vs. 31.6 kg/d, $P < 0.06$). Compared with HT, CL cows had a higher percentage of proliferative epithelial cells at −20 d relative to calving (3.3 vs. 1.3%, $P < 0.05$), but there was no difference in labeled stromal cells ($P > 0.1$). We conclude that heat stress abatement during dry period improves milk production in the subsequent lactation possibly by increasing mammary epithelial cell proliferation during the dry period.

Key Words: heat stress, mammary gland, epithelial cell

476 Characterization of bovine glucose transporter 1 kinetics and substrate specificities in *Xenopus laevis* oocytes. P. A. Bentley[1], Y. Misra[1], A. D. Morielli[2], and F.-Q. Zhao*[1], [1]*Lactation and Mammary Gland Biology Group, Department of Animal Science, University of Vermont*, *Burlington,* [2]*Department of Pharmacology, College of Medicine, University of Vermont*, *Burlington*.

Glucose is essential for milk production as it serves as both a substrate for lactose synthesis and as an energy source. Glucose uptake in the bovine mammary gland therefore plays a key role in milk synthesis. Facilitative glucose transporters (GLUTs) mediate glucose uptake in the mammary gland. GLUT1 is the major facilitative glucose transporter expressed in the bovine gland and has been shown to localize to the basolateral membrane of mammary epithelial cells. GLUT1 is therefore thought to play an important role in glucose uptake during lactation. The objective of this study was to determine the kinetic properties of bovine GLUT1 transport using the *Xenopus* oocyte model. Bovine GLUT1 was expressed in *Xenopus* oocytes by microinjection of in vitro transcribed cRNA and was found to be localized to the plasma membrane, which resulted in increased glucose uptake. This bGLUT1-mediated glucose uptake was dramatically inhibited by specific facilitative glucose transport inhibitors, cytochalasin B and phloretin. Kinetic analysis of bovine GLUT1 was conducted under zero-trans conditions using radio-labeled 2-deoxy-D-glucose and the principles of Michaelis-Menten kinetics. Bovine GLUT1 exhibited a Km of 7.69 ± 1.7 m*M* for 2-deoxy-D-glucose. Transport by bovine GLUT1 was inhibited by mannose and galactose, but not fructose, indicating that bovine GLUT1 may also be able to transport mannose and galactose. Our data provide insight into potential functional properties of GLUT1 in transporting glucose across mammary epithelial cells for milk synthesis.

Key Words: glucose uptake, milk synthesis, glucose transporter