449 The acute response to milk removal and the long-term response to frequent milking treatment involve distinct mechanisms. E. H. Wall*, J. P. Bond, and T. B. McFadden, *University of Vermont*, *Burlington*.

Frequent milking during early lactation of dairy cows elicits a persistent increase in milk yield. We hypothesized that this response would be associated with increased mammary growth and altered expression of genes involved in the insulin-like growth factor (**IGF**) axis. Four multiparous cows were assigned at parturition to unilateral frequent milking (**UFM**; twice daily milking of the left udder half (**2×**; 0230 and 1430 h), four-times daily milking of the right udder half (**4×**; 0230, 0530, 1430 and 1730 h)). Mammary biopsies were obtained from both udder halves at 5 days in milk (**DIM**) at 0530 h (immediately after 4× glands were milked). Incorporation of [^{3}H]-thymidine into DNA, mammary cell apoptosis, and expression of IGF-1, IGF-1 receptor, and IGF binding protein (**IGFBP**)-1 mRNA were not affected by UFM (*P's* > 0.20), whereas expression of IGFBP-3 was lower in 4× glands ($P \leq 0.06$). Using Affymetrix GeneChip® Bovine Genome Arrays, we identified 84 genes that were differentially expressed in 2X vs. 4X glands ($P < 0.05$). Because biopsies were obtained when glands were at different intervals since the previous milking, our results included both the acute mammary response to milk removal and the long-term response to UFM treatment. A second experiment was designed to distinguish the mechanisms involved in each of these responses. Mammary biopsies were obtained from UFM cows (n = 5) at 0500 h, when udder halves were at the same interval since the previous milking. Mammary cell apoptosis and expression of the genes listed above were not affected by UFM (*P's* > 0.20). We identified an intersection of 13 genes that were differentially expressed in both experiments, consistent with their involvement in the persistent milk yield response. In contrast, the remaining 71 genes appear to be involved in the acute mammary response to milk removal. We conclude that frequent milking does not alter mammary growth or genes of the IGF axis at 5 DIM. Moreover, the acute mammary response to milk removal and the long-term response to frequent milking appear to involve separate mechanisms.

Key Words: Frequent Milking, Gene Expression

450 The effects of increased milking frequency during early lactation on metabolism and mammary cell proliferation in Holstein cows. F. Soberon*[1], J. L. Lukas[1], M. E. Van Amburgh[1], A. V. Capuco[2], and T. R. Overton[1], [1]*Cornell University*, *Ithaca, NY*, [2]*Bovine Functional Genomics Laboratory, USDA-ARS*, *Beltsville, MD*.

Primiparous (n=30) and multiparous (n=30) cows were assigned randomly at calving to one of 2 treatments to evaluate metabolic and mammary cell responses to increased milking frequency (IMF). Controls were milked twice daily (2X) for 119 d and the IMF group was milked four times daily (4X) from d 2 until d 21 postpartum and 2× from d 22 until d 119. Early lactation IMF did not affect overall milk yield (42.0 vs. 40.6 kg/d; $P > 0.10$). An interaction of treatment by week ($P < 0.01$) indicated that IMF cows yielded 4.8 kg/d more milk than controls during wk 2 and 3 but yields were comparable thereafter. Although there was no interaction of treatment and parity ($P = 0.31$), a trend ($P = 0.08$) for an interaction of treatment, parity, and week suggested that overall responses were larger in primiparous cows. When cows subjected to mammary biopsies were excluded, an overall interaction of treatment by parity existed ($P < 0.01$) such that milk yield was increased throughout the study period by IMF (40.1 vs. 34.2 kg/d) in primiparous but not multiparous cows (47.1 vs. 50.1 kg/d). Plasma nonesterified fatty acids were elevated in multiparous (679 vs. 468 μEq/L) but not primiparous (583 vs. 562 μEq/L) cows subjected to IMF (treatment by parity, $P < 0.05$). Plasma β-hydroxybutyrate was not affected by IMF. Mammary tissue was biopsied from a subset of cows (n=8 per parity group and treatment) at calving, 21 d postpartum, and 75 d postpartum and used for immunohistochemical localization of Ki-67. A treatment by day interaction ($P = 0.03$) indicated that IMF cows had fewer labeled epithelial cells than controls at d 21 (0.31 vs. 0.82%) but more labeled epithelial cells at d 75 (1.37 vs. 0.65%). Overall, results suggest that metabolic health may influence the milk yield response of cows to early lactation IMF. Further analysis of mammary cellular dynamics is needed to determine specific mechanisms for milk yield responses to early lactation IMF.

Key Words: Milking Frequency, Mammary Gland

451 Effects of serotonin receptor antagonists on milk protein gene expression in primary bovine mammary epithelial cells. L. Hernandez*[1,2], J. Collier[1], L. Baumgard[1], N. Horseman[2], and R. Collier[1], [1]*University of Arizona*, *Tucson*, [2]*University of Cincinnati*, *Cincinnati*.

Serotonin (5-HT) is proposed to be a feedback inhibitor of lactation (FIL) in the bovine specifically, decreasing α-lactalbumin and β-casein mRNA expression in primary bovine mammary epithelial cells (BMEC) and milk yield in lactating dairy cows. However, there are 7 5-HT receptor subtypes currently identified and it is critical to determine which one(s) is present in bovine mammary tissue. Therefore, we investigated effects of selective 5-HT receptor antagonists on milk protein mRNA expression in BMEC. Cells were cultured in collagen gels, proliferated for 7 d in a serum-free (SF) medium containing IGF-I, EGF, and insulin and then treated with a SF lactogenic medium containing IGF-1, hydrocortisone and PRL with gel release for 48 h. Treatments included 0.1, 1.0 and 10 μM of the 5-HT receptor antagonists; SB-224289 (1b antagonist), ritanserin (2a antagonist), SB-204741 (2b antagonist), and pimozide (7 antagonist), and 0.0001, 0.01 and 1.0 μM of SB-204070 (4 antagonist). Lactogenic medium with gel release alone served as control (CTRL). Exogenous SB-224289, at a dose of 1.0 μM and all doses of ritanserin up-regulated α-lactalbumin mRNA expression ($P<0.05$) relative to the CTRL. The 1.0 μM of ritanserin had the greatest effect on α-lactalbumin mRNA expression, causing a 16-fold increase relative to the CTRL ($P<0.001$). All other treatments did not affect α-lactalbumin mRNA expression ($P>0.05$). The 0.1 μM doses of SB-224289, SB-204070 and pimozide increased β-casein mRNA expression ($P<0.05$) relative to the CTRL. The 1.0 μM dose of ritanserin increased β-casein mRNA expression relative to the CTRL ($P<0.05$). Antagonism of the 5-HT1b and 5-HT2a receptors increased α-lactalbumin mRNA expression. Furthermore, antagonism of the 5-HT1b, 5-HT4 and 5-HT7 increased β-casein mRNA levels. These results indicate there are multiple 5-HT receptor subtypes present in bovine mammary epithelial tissue and further support the involvement of the serotonergic system as a FIL in the bovine.

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Key Words: Serotonin, FIL, Serotonin Receptors

452 Effect of experimental tight junction opening on milk production. H. Ben Chedly*[1], M. Boutinaud[1], P. Lacasse[3], R. Bounhamous[2], and P.-G. Marnet[2,1], [1]*INRA, UMR 1080 Production du Lait*, *Saint Gilles, France*, [2]*Agrocampus Rennes*, *Rennes, France*, [3]*AAFC-Dairy and Swine R&D Centre*, *Sherbrooke, QC, Canada*.

In situations where milk yield is decreasing rapidly, an opening of tight junctions between mammary epithelial cells (MEC) is often observed. The goal of this experiment was to determine if an experimental induction of tight junction opening by chelation of milk calcium by EGTA or citrate affects milk production, MEC activity and MEC apoptosis. Three hours after milking, 4 dairy goats received a 250 mL intra-mammary infusion of EGTA (115 mM) in the right gland and an equal volume of physiologic saline in the left gland. Similarly, 4 other goats received a 32 mL infusion of citrate (50 mM) in the right gland and lactose (300 mM) in the left gland. The procedure was repeated for 4 consecutive milkings. Mammary biopsies were performed before the milking following the 4th infusion. EGTA infusion induced a decrease in milk free calcium concentration (55%; P<0.01) associated with an increase in milk serum albumin concentration (6 fold; P<0.01), milk Na/K ratio (P=0.02) and blood lactose (P=0.02) indicating the opening of tight junctions. In contrast, the increase in milk serum albumin concentration caused by lactose infusion was larger than for citrate, (P=0.02) suggesting that lactose caused tight junction opening. Tight junction opening in EGTA and lactose treated udder was associated to a decrease in milk yield (respectively 30%; P=0.03 and 25%; P=0.01). Gene expression of proteins involved in milk synthesis or cell apoptosis were quantified by qRT-PCR. Induction of tight junction opening was associated with lower α-lactalbumin (P=0.08 and P=0.03) and higher pro-apoptotic protein Bax (P=0.10 and P=0.02) mRNA levels for EGTA and lactose infusions, respectively. Higher TUNEL labeling were observed in EGTA (P=0.10) and lactose (P=0.02) infused glands which confirmed cell death induction. In conclusion, the lower MEC activity and higher MEC apoptosis were involved in the milk yield decrease consecutive of tight junction opening.

Key Words: Tight Junction, Mammary Gland, Apoptosis

453 Suppression of bovine αS1-casein gene expression during involution of the mammary gland is associated with increased DNA methylation at a STAT5-binding site in the αS1-casein promoter. K. Singh*[1], K. Swanson[1], C. Couldrey[1], H.-M. Seyfert[2], and K. Stelwagen[1], [1]*AgResearch Ltd, Ruakura Research Centre*, *Hamilton, New Zealand*, [2]*Research Institute for the Biology of Farm Animals (FBN)*, *Dummerstorf, Germany*.

Milk protein gene expression in the mammary gland is regulated in a tissue-specific and temporal manner. During lactation, the transcription factor STAT5 is activated in response to prolactin and stimulates expression of milk protein genes by binding to target sequences on the promoters. At the onset of mammary involution there is a rapid decline in milk protein gene expression. DNA methylation is an epigenetic event which results in the silencing of gene expression. The DNA methylation at a functional STAT5-binding site in the promoter of the bovine αS1-casein gene is associated with the shutdown of casein synthesis during acute mastitis. To elucidate the silencing mechanism of αS1-casein during bovine mammary gland involution, the DNA methylation status across the promoter was examined in alveolar tissue obtained from non-pregnant cows in mid-lactation slaughtered at 0, 6, 12, 18, 24, 36, 72 and 192h (n = 6 per group) after the last milking. Quantitative real time RT-PCR analysis showed αS1-casein mRNA levels were down-regulated 6.6-fold (P < 0.01) by 24 h post milking, relative to 6 h and expression continued to decrease (P < 0.001) until 192 h after the last milking. A quantitative MassARRAY methylation analysis (www.sequenom.com) showed 2 of the 3 CpG sites at the STAT5-binding site approximately -10kbp upstream of the αS1-casein-encoding gene, had increased methylation levels. One site was increased (P < 0.05) from 21% to 44% at 36 h post milking compared to 18 h. The other site was increased (P < 0.05) from 34% to 60% by 192 h post milking compared to 18 h post milking. Results suggest that alterations in methylation status at CpG sites at the functional STAT5 binding site in the far upstream promoter are associated with the silencing of the αS1-casein gene during involution.

Key Words: DNA Methylation, Bovine αS1-Casein, Mammary Involution

454 Extending lactation in pasture-based dairy cows: Effect of genetic strain and diet on hormones and metabolites. J. Kay*, P. Aspin, C. Phyn, J. Roche, D. Clark, and E. Kolver, *DairyNZ*, *Hamilton, New Zealand*.

The effect of genetic strain and diet on plasma hormone and metabolites was investigated in pasture-based dairy cows milked continuously for up to 670 d. Fifty-six genetically divergent New Zealand (NZ) and North American (NA) Holstein-Friesians (HF) grazed pasture and were offered 0, 3, or 6 kg concentrate DM/cow/d for 604 ± 8 DIM. Weekly blood samples collected from individual cows during wk 1-10 and wk 47-63 (extended lactation) were analyzed for NEFA, BHBA, insulin, glucose, leptin, growth hormone (GH) and IGF-I. Data were averaged for each period and analyzed by REML with genetic strain, diet and interactions as fixed effects and cow as random effect. In the first 10-wks postpartum there was no strain effects on BHBA, leptin, insulin or glucose, however NA HF had greater (P<0.05) NEFA and GH and lower (P<0.05) IGF-I compared with NZ HF. Plasma NEFA and GH were also greater (P<0.05) in NA HF during the extended lactation period, but IGF-I was not affected by strain, and BHBA and glucose were greater (P<0.05) in NZ HF. During the extended lactation period, leptin was greater (P<0.01) and insulin tended to be greater (P=0.07) in NZ HF. A strain by diet interaction (P<0.01) revealed that this effect was due to a greater leptin and insulin content in NZ HF fed the highest concentrate level than either of the other 5 treatments that did not differ from each other. Supplementation during the first 10-wk postpartum linearly increased (P<0.01) glucose and decreased GH, but did not alter BHBA, NEFA, leptin, insulin or IGF-I. During the extended lactation period, NEFA and IGF-I were unaltered and glucose and GH followed the same pattern with diet. However, BHBA increased linearly (P<0.01) with increasing supplementation. These data are consistent with the previously reported greater milk production and BCS loss in NA HF compared to NZ HF and indicate potential strain differences in recoupling of the somatotropic axis and energy partitioning. These results have implications for breeding and diet management during an extended lactation.

Key Words: Extended Lactation, Genetic Strain, Pasture-Based

455 Effect of the prolactin-release inhibitor Quinagolide on dairy cows. P. Lacasse*[1], V. Lollivier[2], R. M. Bruckmaier[3], Y. R. Boisclair[4], G. W. Wagner[5], and M. Boutinaud[2], [1]*Dairy and Swine R&D centre*, *Sherbrooke, QC, Canada*, [2]*INRA, Agrocampus Rennes*, *St-Gilles, France*, [3]*University of Bern*, *Switzerland*, [4]*Cornell University*, *Ithaca, NY*, [5]*University of Western Ontario*, *London, ON, Canada*.

In most mammals, prolactin (PRL) is essential to maintain lactation. Nevertheless, short term suppression of PRL by bromocriptine has produced inconsistent effects on milk yield in cows and goats. To asses the effect of long term inhibition of PRL release in lactating dairy cows, five Holstein cows in early lactation received daily i.m. injection of 1 mg of Quinagolide for 9 weeks. Four control cows received the vehicle (water). During the last week of treatment, one half-gland was milked once (1X) and the other twice a day (2×). Blood samples were harvested at milking on wks -1, 1, 4 and 8 and mammary biopsies were taken at wks -1, 4 and 8. Daily injections of Quinagolide reduced milking-induced PRL release ($P<0.05$) but not basal PRL. Quinagolide induced a faster decline in milk production ($P<0.05$). During week 9, the inhibiton of milk production by Quinagolide was maintained in half-gland milked 2X ($P<0.05$) but not in half-gland milked 1X. Milk production was significantly correlated with the quantity of PRL released at milking ($P<0.01$) but not with its basal level. Quinagolide did not affect the release of oxytocin at milking. The concentration of IGF-1 was not affected by treatment or correlated with milk production. Serum concentration of leptin and of the calciotropic hormone stanniocalcin were not affected by the treatment. Levels of κ-casein ($P<0.05$) and α-lactalbumin ($P=0.06$) mRNA determined by qRTPCR were lower in mammary biopsies of Quinagolide-treated cows at wk 4. Western blotting and immunohistological determination of tissue harvested at wk 8 have shown that the protein PCNA was less abundant ($P<0.05$) and present in a lower number of cell ($P<0.05$) in Quinagolide-treated animals, suggesting a reduction in cell proliferation. In conclusion, chronic administration of the prolactin-release inhibitor Quinagolide reduces milk production in dairy cows.

Key Words: Leptin, Stanniocalcin, Persistency

456 Effect of estradiol cypionate injected at dry-off on lactose concentrations in bovine plasma. M. S. Gulay*[1], M. J. Hayen[2], H. H. Head[2], and K. C. Bachman[2], [1]*Mehmet Akif Ersoy University*, *Burdur, Turkey*, [2]*University of Florida*, *Gainesville*.

Elevation of lactose in plasma of dairy cows because of mammary cell tight junction leakage serves as an indicator of the onset of mammary tissue involution. Therefore, objective of this study was to determine the effect of supplemental estradiol cypionate (ECP) at dry-off on concentrations of plasma lactose in Holstein cows. Thirty-two Holstein cows were assigned to four dry-off treatment groups (n= 8/group; 30 d dry, 30 d dry +ECP, 60 d dry, and 60 d dry +ECP). Single injections of ECP (15 mg) in cottonseed oil or equal quantities of cottonseed oil carrier were administered intramuscularly at dry-off to equal numbers of cows given 30 or 60 d dry periods. Blood samples were collected from the coccygeal vein 24 h prior to dry-off, at dry-off, and throughout the subsequent 48 h (12, 8, 4, 4, 4, 4, 4 and 8 h intervals) to monitor plasma lactose. Actual dry period lengths for cows in 30d, 30d+ECP, 60d, and 60d+ECP were 26.8, 29.1, 54.2, and 59.1 d, respectively. No differences in mean milk yields at dry off for the four groups were observed (30d=16.1±4.3, 30d+ECP=14.3±4.3, 60d=14.0±4.2, 60d+ECP=14.4±4.4 kg/d: $P=0.47$). Similarly, no significant effect of ECP on overall concentrations of plasma lactose was detected (30d=282.7±52.1, 30d+ECP=189.5±48.9, 60d=191.0±51.6 and 60d+ECP=196.6±52.9 μM; $P=0.62$). Lactose concentrations increased and were greatest in blood plasma at 20 h after the injection and final milk removal. At 40 h, concentrations approached those observed prior to dry-off. Plasma lactose concentrations at 20 h were positively correlated with milk yield at dry-off ($R^2=0.52$). The temporal profiles of lactose in blood plasma were similar across all treatments and indicated that supplementing 15 mg ECP at dry-off did not alter markedly the course of tight junction leakage that occurs in mammary epithelial tissue when milk removal is discontinued at dry-off.

Key Words: Dry Period, Estrogen, Plasma Lactose

457 Heat stress abatement for dry cows: Does cooling improve transition into lactation? B. C. do Amaral*[1], J. Hayen[1], E. E. Connor[2], S. Tao[1], and G. E. Dahl[1], [1]*University of Florida*, *Gainesville*, [2]*USDA-ARS*, *Beltsville, MD*.

Environmental factors, especially temperature and photoperiod, influence health and productivity of dairy cows during lactation, possibly via similar physiological effects. For example, heat stress is a critical component of lowered milk yield during summer. Long days improve yield during lactation, while short days improve health and subsequent performance in dry cows. The objective of the study was to evaluate the effects of heat stress prepartum under controlled photoperiod on lactation performance and hepatic metabolic gene expression of periparturient Holstein cows (n=16). Cows were dried off 46 d before expected calving date and assigned to treatments by mature equivalent milk production. The treatments were: 1) Heat stress (HT) and 2) Cooling (CL). Both treatments had a photoperiod of (14L:10D). Rectal temperature was measured 2x daily during the dry period. After calving, cows were housed in a free stall barn with cooling, and milk yield was recorded daily up to 42 DIM. Daily DMI was measured from -35 to 42 d relative to calving. Liver biopsies were collected at dry off, -20, +2, and +20 d relative to calving for cows on HT (n=5) and CL (n=4) to measure mRNA expression of *prolactin receptor (PRL–R), suppressors of cytokine signaling (SOCS1, 2, and 3), CAV1, IGFII, IGFBP5*, a key transcription factor in lipid biosynthesis (*SREBP1-c*), and enzymes of lipid metabolism (*FASN, ACACA, ACADVL, LCAT, CPT1A, and ACOX*) by real-time qPCR. HT cows had greater afternoon rectal temperatures (39.2 vs. 38.8°C) and decreased DMI prepartum (12.0 vs. 14.1 kg/d) and milk yield postpartum (25.4 vs. 33.3 kg/d) compared with CL cows. Relative to CL cows, hepatic mRNA expression of *SOCS2* and *IGFBP5* was down-regulated in HT cows. Expression of *ACADVL* was up-regulated in CL cows at d +2 but down-regulated at d +20 relative to HT cows. These results suggest that heat stress abatement in the dry period improves subsequent lactation, possibly through *SOCS-2* and its regulation of hepatic lipid metabolism.

Key Words: Heat Stress, Dairy Cattle, Gene Expression