462 Late gestation and advanced lactation at cessation of milking do not delay mammary epithelial apoptosis in dairy cattle. E. L. Annen*[1], A. V. Capuco[2], P. C. Gentry[1], L. H. Baumgard[1], and R. J. Collier[1], [1]*University of Arizona, Tucson,* [2]*USDA-ARS, Bovine Functional Genomics Lab, BARC, Beltsville, MD.*

Advanced pregnancy and concurrent lactation until cessation of milking are two factors thought to slow mammary involution in dairy animals. Our objective was to characterize the temporal pattern of apoptosis in the bovine mammary gland following milk stasis. Serial mammary biopsies were performed on 11 Holstein cows during late lactation and the dry period. Cows were dried-off 60d before expected calving. Mammary biopsies were taken at -5, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, and 21d relative to cessation of milking. Tissues were fixed in neutral buffered formalin, and paraffin sections were subjected to TUNEL assay for immunohistochemical detection of apoptotic cells. The incidence of mammary epithelial cells undergoing apoptosis was lowest in lactating tissue, peaked ($P< 0.0001$) 2d following cessation of milking and then declined through d8. At d2, the incidence of apoptosis was 4 to 8-fold greater than that in lactating tissue. By d8, the number of apoptotic mammary epithelial cells did not differ ($P > 0.05$) from pre-stasis values. Rodents and ewes typically initiate apoptosis by 2d post-stasis and reach peak apoptosis at approximately 4d. Because these species are not typically in advanced gestation and lactation when milk removal ceases, we hypothesized that onset of apoptosis would be slower and incidence of apoptosis reduced in cows as compared to sheep and rodents. Our data indicate that onset of epithelial apoptosis in cows is rapid but transient and alveolar integrity is maintained throughout the 21d post dry-off. Further, a second wave of apoptosis involving intralobular stromal elements appears to initiate at d4 and peak at d5 ($P<0.05$). The balance between apoptosis and cell proliferation during this time frame is currently under investigation. Data are consistent with rapid initiation of tissue remodeling of both epithelium and stroma during the dry period of dairy cows.

Key Words: Mammary apoptosis, Involution

463 Microarray analysis of bovine mammary gene expression following abrupt cessation of lactation. S. R. Davis*[1], A. J. Molenaar[1], K. Stelwagen[1], T. T. Wheeler[1], C. J. McMahon[1], D. B. Baird[3], H. V. Henderson[1], V. C. Farr[1], L. Good[1], K. Odin[1], K. Singh[1], D. L. Hyndman[2], and T. Wilson[2], [1]*AgResearch Hamilton,* [2]*Dunedin,* [3]*Lincoln, New Zealand.*

This work identified early changes in gene expression triggered by mammary engorgement that lead to apoptosis. Alveolar tissue was obtained from 36 non-pregnant cows in mid-lactation slaughtered at 6, 12, 18, 24, 36 and 72h (n=6/group) after last milking. mRNA was extracted from 6 and 36h tissue using TRIZOL, cDNA prepared and labeled with Cy3 and Cy5 dyes using an Ambion kit. Samples were hybridized to bovine ESTs arrayed on glass slides. Each slide had 23954 ESTs arrayed (including limited replication) representing 16550 ESTs with known SwissProt hits and 6772 with no known hit, selected from mammary and immune bovine libraries. Microarray slides (n=24) were analysed in a "daisy chain" design. Each cow at each time point (6 cows) was compared with 2 cows from the opposite time point along with its dye reversal. Hybridized slides were read on a Packard array reader and images analysed by GenePix software. For each slide log ratio data were normalized using a mixed model with spatial autocorrelation using REML in GenStat. Means and SE of corrected log ratios across the 24 slides were calculated for each EST. Results at 36h showed expression of 5300 ESTs ($P<0.01$) and 3300 ESTs ($P< 0.001$). At $P<0.01$, 941 ESTs changed expression by >50%; at $P<0.001$ the comparable figure was 766. In this latter group 122 ESTs were signal peptide +ve. β-casein and α-lactalbumin were down-regulated (25% and 50%) and lactoferrin up-regulated (80%), with multiple ESTs of each gene exhibiting similar behavior. Northern analysis of major milk protein RNA indicated increased expression of lactoferrin and decreased expression of α-lactalbumin by 24h. Expression of all milk proteins was still apparent at 72h. Early up-regulation of acute-phase proteins was also apparent. Microarray analysis has provided evidence of widespread changes in bovine mammary gene expression occurring within 36h post milking and provides an effective base for testing refined hypotheses.

Key Words: Microarray, Mammary, Involution

464 Evidence of cisternal recoil after milk letdown in the udder of dairy cows. G. Caja*[1], M. A. Ayadi[1], and C. H. Knight[2], [1]*Universitat Autonoma de Barcelona, Spain,* [2]*Hannah Research Institute, UK.*

A delay between activation of the milk letdown reflex and milk evacuation from the udder can negatively affect milk yield. Linzell (1955) was the first to demonstrate back-flux of milk from ducts into alveoli of mice after milk letdown, and here we investigate the possibility that the elastic nature of the udder cistern results in an equivalent phenomenon in dairy cows. Two groups of Holstein cows in early (n=3; 80 DIM, 31 kg/d) and late lactation (n=4; 301 DIM, 17.5 kg/d) were used. Udder cistern size was measured by real time ultrasonography. Left and right front udder quarters were scanned in duplicate at 0, 3, 15, 30 and 60 min after an i.v. oxytocin (OT) injection. For the first udder scan, cows were injected i.v. with an OT receptor blocking agent (Atosiban; 10 μg/kg BW) to prevent spontaneous milk letdown. Cistern measures were repeated for 8 and 16 h milking intervals. Values of cistern area (1.1 to 33.6 cm^2) differed according to stage of lactation ($P<0.01$), time after OT injection ($P<0.001$) and milking interval ($P<0.01$). Average cistern area increased dramatically (93%) at 3 min after OT injection at which time the cistern reached its maximum distension (16.8 vs 8.7 cm^2; $P<0.001$), and decreased slowly thereafter (14.6, 13.5 and 12.8 cm^2 at 15, 30 and 60 min, respectively). The decrease in cistern size was significant at 15 min and later time points ($P<0.05$) but not at earlier time points. The 0 and 3 min data provide clear evidence of milk letdown. The decrease in cistern size thereafter provides the first report, to our knowledge, documenting the return of milk to the ductal and alveolar compartments of the cow udder following the end of milk letdown. We term this cisternal recoil. The process will result in a mixing of milk that has been stored for some time with freshly synthesized milk. Given the presence in milk of putative inhibitory bioactive factors, this could have consequences for further secretory activity.

Key Words: Residual milk, Cisternal milk, Udder physiology

465 Kinetics of glucose transport and metabolism in lactating bovine mammary glands measured in vivo with a paired nutrient/indicator dilution technique. F. Qiao*, C. Xiao, D. R. Trout, and J. P. Cant, *University of Guelph, Ontario, Canada.*

Twenty-four paired glucose and extracellular indicator (p-aminohippuric acid) venous dilution curves across intact bovine mammary glands were obtained from bolus injections into the external iliac artery to measure kinetics of glucose transport and metabolism. A compartmental capillary, convolution integration model was used to interpret the curves. Four different capillary submodels, describing hypothetical mechanisms of glucose transport and metabolism, were expressed in ordinary differential equations and fitted to the observed curves by an iterative approach to least-squares. Submodel I, assuming first-order unidirectional uptake and metabolism of glucose, was unable to fit the peak and tail of glucose dilution curves ($r^2 = 0.96$). Submodel II, considering transport as a first-order bidirectional process, yielded good fits ($r^2 = 1.00$), but errors of estimation of the transport parameter were high (428 times the estimate, on average). Transport rate constants were 5 to 50 times greater than metabolism rate constants and, when expressed as clearance values, 5 times greater than blood flow rates. Submodel III assumed instantaneous mixing between extra- and intra-cellular glucose distribution spaces and first-order kinetics of metabolism. This submodel yielded $r^2 = 0.99$ and low errors of parameter estimation (<32% of estimate). The metabolism rate parameter $k_c = 0.406 \pm 0.083$ min^{-1} was not different from that calculated from background arterial and venous glucose concentrations ($k_{c\_bg} = 0.404 \pm 0.119$ min^{-1}). Assuming Michaelis-Menten kinetics of glucose metabolism in Submodel IV did not improve goodness-of-fit and parameters were less identifiable. It was concluded that glucose is rapidly translocated into an intracellular space that is 34% of intracellular volume, that once glucose enters the cytosol proper where metabolism occurs, there is negligible efflux out of the cell, and that glucose sequestration follows first-order kinetics between 0 and 5 mM extracellular glucose.

Key Words: Glucose transport, Mammary glands, Indicator dilution

466 The effect of phenotypic selection for milk production on hepatic expression of prolactin receptor. P. H. Luimes*[1], E. H. Beaupre[1], J. H. White[1], W. J. Weber[2], H. Chester-Jones[2], L. B. Hansen[2], B. A. Crooker[2], and J. R. Knapp[1], [1]*University of Vermont, Burlington,* [2]*University of Minnesota, St. Paul.*

The effect of phenotypic selection for milk production on hepatic expression of the prolactin receptor gene was evaluated in a line of dairy cattle selected solely for milk production. Selection since 1964 resulted in increases of approximately 5500 kg of milk per lactation as compared to a control line. Liver biopsies were taken at -14, 14, 21 and 70 days relative to parturition (5 cows from each line). Total RNA was isolated and purified. Northern blots of the samples were hybridized with an RNA probe homologous to the extracellular portion of the bovine prolactin receptor. Normalization of RNA loading on the membranes was determined by hybridizing with an 18S RNA probe. RNA expression was quantified as relative pixel intensity. The selected cows had approximately 42% greater expression of prolactin mRNA at both 14 days prior to and after calving relative to the control cows ($P < 0.05$). These differences disappeared by day 21 such that, at days 21 and 70, there were no differences in prolactin receptor mRNA expression between selected and controls cows. Little is known about the importance of prolactin on hepatic tissue metabolism in ruminants. These data suggest the prolactin receptor has a role in regulating hepatic metabolism in support of lactation, though the mechanism by which this occurs is unknown.

Key Words: Prolactin receptor, Milk yield, Phenotypic selection

467 Quantitative analysis of estrogen-related receptor α, estrogen receptor α and estrogen receptor β mRNAs throughout bovine mammary gland development. E. E. Connor*[1], A. V. Capuco[1], T. S. Sonstegard[1], A. F. Mota[1], D. L. Wood[1], W. Garrett[1], G. L. Bennett[2], and J. Williams[3], [1]*USDA-ARS, Beltsville, MD,* [2]*USDA-ARS, Clay Center, NE,* [3]*Roslin Institute, Roslin, Midlothian, Scotland.*

The estrogen-related receptor α (ESRRA) belongs to the steroid hormone receptor family and is thought to function in regulation of estrogen-responsive genes including lactoferrin and medium-chain acyl coA dehydrogenase. The role of ESRRA in bovine mammary gland development and function is unknown. Expression of ESRRA mRNA was characterized in mammary parenchyma obtained from multiple stages of bovine mammary gland development in relation to estrogen receptor α (ESR1) and estrogen receptor β (ESR2) using quantitative real-time RT-PCR. Stages of development included prepubertal heifers, pregnant heifers, lactating non-pregnant cows, lactating pregnant cows and non-lactating pregnant cows (n = 2 to 3 animals/stage). In addition, the *ESRRA*, *ESR1* and *ESR2* genes were mapped to chromosomes 29, 9 and 10, respectively by linkage and radiation hybrid mapping. Results indicated expression of ESRRA mRNA was greatest in mature cows, regardless of state of pregnancy or lactation and ranged from 20 to 120-fold more than ESR1 and ESR2 transcripts. Expression of ESR2 mRNA was low across all physiological stages and generally less than ESR1 and ESRRA. In pregnant heifers (approx. 100-200 d of pregnancy), levels of all three transcripts were at their lowest or non-detectable. Similar decreases during pregnancy have been reported for mice and may indicate down-regulation by high levels of estradiol during this developmental period. In prepubertal heifers, ESR1 mRNA was at its maximal level of expression but was half as abundant as ESRRA. Our results demonstrate expression of ESRRA, ESR1 and ESR2 mRNAs in bovine mammary gland and suggest a functional role of ESRRA in mammary gland development and lactation.

Key Words: Estrogen receptors, Mammary gland, Bovine

468 Effects of varying energy intakes on estrogen receptor, cell proliferation, and tissue composition in mammary tissue of pre-pubertal heifers. J. W. Forrest*[1], R. M. Akers[1], R. E. Pearson[1], E. G. Brown[2], M. J. VandeHaar[2], and M. S. Weber Nielsen[2], [1]*Virginia Tech, Blacksburg,* [2]*Michigan State University, East Lansing.*

Our objective was to determine how varying energy intakes between 2 and 14 wk of age affect mammary parenchymal development. At 2 wk of age, Holstein calves were assigned to 1 of 4 treatments (HH, HL, LH, and LL) with 2 levels of energy intake (High or Low) and 2 periods of growth (2 to 8 and 8 to 14 wk of age). Period 1 gains were 379 and 666 g/d for L and H calves, respectively. Period 2 gains were 439 and 1095 g/d for L and H calves. At 14 wk, parenchyma at the stromal interface (I), mid-gland (M), and above the cistern (C) were collected, fixed, and embedded in paraffin. Digital images of stained sections were used to determine tissue composition (% epithelial, lumenal, and stromal area). Immunocytochemistry revealed estrogen receptor and Ki67 (nuclear proliferation antigen) positive cells (ER^+ and $Ki67^+$). Epithelial area was not affected by treatment (18.0 to 20.9%). However, lumenal and stromal areas were 3.5 ± 1.4% lower ($p<0.01$) and 4.0 ± 1.7% higher ($p<0.01$), respectively, in LL+LH heifers compared to HH+HL heifers. Zone I contained 3.9 ± 1.5% less ($p<0.01$) lumen and 5.3 ± 1.8% more ($p<0.005$) stroma than zones M and C. Treatment did not alter percent ER^+ epithelial cells, but there was a tendency ($p<0.2$) for zones M and C to have more ER^+ cells than zone I. Percent ER^+ cells in subtending ducts (SUBs) and terminal ductular units (TDUs) was 47.2 ± 1.2% and 53.2 ± 1.4%. Percent proliferating cells tended ($p<0.2$) to be higher in zone I compared with zones M and C. $Ki67^+$ labeling in TDUs and SUBs was 2.1 ± 0.8% ($p<0.01$) and 1.4 ± 0.7% ($p<0.05$) higher for LL+LH heifers compared with HH+HL heifers. Percent $Ki67^+$ cells for SUBs and TDUs was 4.4 ± 0.5% and 5.1 ± 0.5%, respectively. A high rate of gain between 2 and 8 wk of age resulted in greater lumenal area but reduced cell proliferation in mammary parenchyma at 14 wk of age. Positive effects of a reduced rate of gain on cell growth became evident only after 2 months of age.

Key Words: Heifers, Mammary, Calves

Physiology: Gamete physiology

469 Combining *in vitro* embryo production and sexed semen technologies. R. D. Wilson*, K. A. Weigel, P. M. Fricke, M. L. Leibfried-Rutledge, D. L. Matthews, and V. R. Schutzkus, *University of Wisconsin - Madison, Madison, WI.*

The objective of this research was to explore the synergy between sexed semen and *in vitro* embryo production and to assess costs and benefits of these technologies on commercial farms. Genetically superior cull cows were used as donors, and ovaries were collected via colpotomy or at the time of slaughter. Oocytes were aspirated from the ovaries, fertilized 20-24 hours later, and matured to the blastocyst stage. Embryos were transferred into recipient cows and heifers on the same farms. Seven Wisconsin herds have participated thus far, and 154 embryos have been produced from 55 donor cows. Sexed semen from three Holstein sires was used. On average, 2.8 ± 3.3 transferable embryos were created per donor. Individual farms ranged from 1.5 to 4.3 embryos per donor. Recipient data revealed interesting trends. Recipient cows that showed standing estrus had a conception rate of 0.16 ± 0.37, while those resulting from a synchronization program had a mean conception rate of 0.21 ± 0.41. Recipient heifers (all with standing estrus) had a mean conception rate of 0.50 ± 0.52. Interestingly, recipients that were synchronized to ovulate one day later (than for conventional embryo transfer) had slightly higher conception rates than other recipients in both cows (0.21 ± 0.41 vs. 0.19 ± .40) and heifers (0.67 ± 0.52 vs. 0.38 ± 0.52). These results, although preliminary, suggest that low cost in vitro embryo production may have promise as an early system for utilizing sexed semen in dairy cattle breeding programs.

Key Words: In vitro production, Sexed semen

470 Timed insemination of superovulated heifers with sexed sperm. J. L. Schenk*[1], W. B. Henderson[2], and G. E. Seidel, Jr.[3], [1]*XY, Inc.,* [2]*Cyagra/EmTran,* [3]*Colorado State University.*

The objective was to study production of transferable embryos in superovulated Holstein heifers following a fixed-time single insemination with sex-sorted (2×10^6 or 20×10^6) or non-sexed (40×10^6) cryopreserved sperm. Sexed inseminates were enriched for the X-chromosome (90%) by flow sorting using a MoFlo® SX sperm sorter. Three subgroups of 12 heifers each were allocated to one of 3 Holstein bulls. Each heifer within a subgroup was inseminated with semen from each treatment. Heifers received a CIDR® on Day 0 and were superstimulated starting