Lactation Biology Symposium: Refining the Old to Answer the New—Moving Approaches Forward to Study Mammary and Lactation Physiology

299 Determinants of milk production: Understanding population dynamics in the bovine mammary epithelium. A. V. Capuco*, Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD.

The mammary gland undergoes distinct periods of growth, development and secretory activity. During a bovine lactation, a gradual decrease in number of mammary epithelial cells largely accounts for the decline in milk production with advancing lactation. The net decline in cell number (~50%) is due to apoptotic cell death, but is accompanied by cell renewal. Though the rate of cell proliferation is slow, by end of lactation most cells in the gland were formed after calving. Typically milking is terminated when cows are in the final 2 mo of pregnancy. This causes regenerative involution, wherein there is extensive cell replacement and mammary growth. We hypothesized that replacement of senescent secretory cells and progenitor cells during the dry period increases milk yield in the next lactation. Analysis of global gene expression revealed networks and canonical pathways during regenerative involution that: support cell turnover and mammary growth, that are consistent with oxidative stress, mitochondrial dysfunction and ER-stress, as well as processes that ameliorate those effects, immune responses consistent with influx of neutrophils, macrophages and lymphocytes, and processes that support mammary differentiation and lactogenesis. Data also suggest that replication of stem/progenitor cells occurs during the dry period. Relying on long-term retention of bromodeoxyuridine-labeled DNA, we identified putative bovine mammary stem cells. These label retaining epithelial cells (LREC) are in low abundance within mammary epithelium (<1%), are predominantly estrogen receptor-negative and localized in a basal or suprabasal layer of the epithelium. Analyses of gene expression in laser-microdissected LREC are consistent with the concept that LREC represent stem cells and progenitor cells, which differ in properties and location within the epithelial layer. We identified potential markers for these cells and have increased their number by infusing xanthosine through the teat canal of prepubertal heifers. Altering population dynamics of mammary stem/progenitor cells during the mammary cycle may be a means to increase efficiency of milk production.

300 Studying hormonal regulation of mammary gland homeostasis. N. D. Horserman*, University of Cincinnati, Cincinnati, OH.

Homeostasis during lactation is a special case in which the unit of homeostatic regulation is not a single organism, but rather a dyad comprising the mother and her offspring (the mother–infant dyad). This dyadic arrangement is not trivial. The familiar laboratory mouse model system includes a mother and litter of infants whose mass can easily exceed the mass of the mother. Some physiological variables, such as body temperature, can remain under conventional homeostatic control within the individual. But other variables such as milk secretion, appetite, and calcium metabolism must come under control of the mother–infant dyad. Prolactin is the primary systemic component of mammary homeostasis. Suckling inhibits dopamine, which induces reflex prolactin secretion in response to nursing. Other factors, such as oxytocin and endorphins increase prolactin output, which sustains enhanced prolactin throughout lactation. In addition to the systemic homeostasis, local homeostatic mechanisms control mammary gland functions. The sophistication of intramammary homeostatic mechanisms is perhaps best illustrated in kangaroos in which individual mammary glands produce different volumes and compositions of milk because they are being nursed by offspring of different ages. The millions of alveolar sacs that comprise the mammary glands are capable of independently regulating their milk outputs in response to local conditions. Most obviously, each alveolus must regulate its secretory output in response to the degree of filling. Serotonin was discovered as a primary regulator of mammary homeostasis. Serotonin synthesis is in the mammary epithelium is elevated during lactation, and increases during milk stasis. Two important functions have been attributed to the intramammary serotonin system. First, when alveolar spaces are filled with milk serotonin inhibits milk secretion and causes tight junction opening. Ultimately, this feedback system induces early phases of involution. Second, serotonin induces secretion of parathyroid hormone-related peptide (PThrP), which is the primary regulator of calcium homeostasis for the mother–infant dyad. These 2 intramammary homeostatic responses are mediated by different receptors. Feedback inhibition of milk secretion is mediated by 5-HTR7, which is autoinhibited and requires sustained occupation to become activated. PThrP secretion is mediated by 5-HTR2B receptors, which are activated at tonic levels of serotonin.

Key Words: serotonin synthesis, lactation, mammary homeostasis

301 Delayed response of xanthosine on goat mammary gland: Quantification of stem/progenitor cells, differentiation and proliferation markers, and milk production in next lactation. T. P. Kaur*, R. Verma1, S. Choudhary1, R. Udehiya3, S. Kaswan3, and R. K. Choudhary1, 1School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India, 2Department of Animal Nutrition, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India, 3Department of Veterinary Surgery and Radiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.

The intramammary infusion of xanthosine (XS) has effects on mammary glands in altering stem/progenitor cell population and possibly on milk production. The aim of this study was to evaluate prolong response of XS on stem/progenitor cells, cell proliferation and differentiation and milk production in following lactation in goats. Six primiparous goats were assigned to the study. Twenty milliliters of 10 mM (2 × 3 d) XS was infused 5 d after parturition into one of the randomly allotted gland (TRT) immediately after morning (0900 h) and evening (2100 h) milking. The other gland of the same animal served as control (CON) with no infusion. Mammary tissues were harvested during the dry period (Mean + SE; 136.8 ± 11.2 d) and processed for histology. In the next lactation, milk production was recorded until 17 weeks. Immunolocalization of alveolar cell differentiation markers (estrogen receptor α (ESR1), progesterone receptor (PR), mucin 1 MUC1), mammary stem cell markers (nuclear receptor subfamily 5 member A member 2 (NR5A2), aldehyde dehydrogenase 1 (ALDH1), fibronectin type III domain containing 3B (FNDC3B), cell proliferation (Ki67) and apoptosis marker (p53) were quantified in terms of number of immune-positive cells of the total cells counted. XS appeared to enhance expression of NR5A2 (7.7 + 0.9 vs. 4.9 + 0.7; Mann-Whitney U test; z = 0.02) and FNDCB (6.9 + 1.2 vs. 3.1 + 0.3; z = 0.004) in relation to CON glands. Expressions of other markers namely ALDH1, ESR1, PR, MUC1, Ki67 and p53 did not differ between TRT and CON glands. Milk yield (mean + SE) of
TRT glands were not different from the CON glands (3462.6 ± 156.1 g/wk vs. 3761.3 ± 205.8 g/wk; paired t-test *P* = 0.28). These results suggest XS may have a prolong effects on increasing stem/progenitor cell population during the dry period but has no response in milk production in the next lactation.

**Key Words:** xanthosine, milk production, stem cell marker

### 302 Deep tissue imaging of lobuloalveolar development in the mouse. C. J. Watson*, Department of Pathology, University of Cambridge, Cambridge, UK.

The pregnancy/lactation/involution cycle of mammary gland development requires the rapid proliferation and subsequent differentiation of both luminal and basal epithelial cells that are presumed to arise from stem cells. To investigate this process, we utilized 2 neutral lineage tracing approaches coupled with new protocols for clearing of mammary tissue to allow deep 3-dimensional (3D) confocal imaging. By labeling stem cells at clonal density we were able to show that individual alveoli arise from unipotent basal and luminal stem cells and that more than one stem cell of each lineage is required to generate an entire alveolus. Furthermore, 3D imaging revealed the presence of multiple binucleate cells in the lactating gland suggesting failed cytokinesis. In addition, deep imaging with antibodies to smooth muscle actin and keratin 14 revealed the distinctive structure of basal cells in alveoli compared with ducts and allowed the collapse of alveoli during involution to be monitored. Interestingly, we discovered an intimate and dynamic association of leukocytes with the epithelium and have analyzed these further by flow cytometry.


Mammary stem/progenitor cells (MaSC) in non-mouse, non-human species are considerably understudied. Interspecies variation in lactation strategies and mammary cancer incidence, combined with the role of MaSC in normal mammary gland function, warrants a comparative study of these cells from various mammalian species. Our laboratory has developed a method for the enrichment of MaSC from virtually any mammal. This method is antibody-independent, uses a small volume of fresh tissue, and consists on propagating freshly isolated mammary epithelial cell migration, and contains factors associated with defense and immunity; all of which are necessary for healing damaged mammary gland tissue. Finally, our group has initiated studies in which to compare the behavior of MDEC from mammary cancer-susceptible and -resistant species, both at baseline as well as in response to pro-tumorigenic stimuli, in order to gain new insight into the mechanisms of breast cancer susceptibility and resistance.

**Key Words:** mammary biology, genomics, epigenetics

### 304 Milk omics: Modern tools to answer ancient questions. D. G. Lemay*1,2, USDA Western Human Nutrition Research Center, Davis, CA, 2University of California-Davis, Davis, CA.

Advances in high throughput biology have revolutionized the study of milk and mammary gland biology. This talk will review applications of genomics, epigenetics, transcriptomics, proteomics, and metagenomics in the field. The availability of mammalian genome sequences, particularly the bovine genome assembly, enabled multi-species comparisons of the genes that are expressed to produce milk. Across mammals, gene duplication and protein sequence variation both contributed to differences in milk composition. ChiP-Seq and transcriptomic data were then leveraged to understand epigenome-wide features involved in milk production. Identification of cis-regulatory elements in the bovine genome using RNA-Seq and ATAC-Seq technology is ongoing. Meanwhile, non-invasive technologies to study mammary biology in humans using RNA-Seq of milk fat RNA have been developed and validated. Whole transcriptome comparisons in a non-human primate model demonstrated that RNA from milk samples provides a more accurate representation of RNA from milk-producing cells than does RNA from whole mammary tissue. Advances in proteomics have expanded the milk proteome from dozens to thousands of unique proteins. The use of RNA-Seq to develop a comprehensive database of protein sequences has the potential to further expand the milk proteome to include isoforms of milk proteins. Finally, microbial metagenomics can be used to survey all microbes in a sample to investigate causal agents in mastitis, manage spoilage organisms, or track antibiotic resistance. Applications of these “omic” technologies in dairy science will be discussed.

Climate change adversely affects the dairy economy as high temperatures and humidity (i.e., heat stress) result in greater incidence of cattle disease and mortality and lower milk yield. Previous research by our group demonstrated consequences of dry period heat stress on cow health and milk production in the subsequent lactation. However, the molecular mechanisms through which dry period heat stress impairs lactation performance have yet to be fully elucidated. In this study, we evaluated the impacts of dry period heat stress on the mammary proteome across the subsequent lactation. During the dry period (~46 d), multiparous Holstein cows were housed in either shaded barns with fans and water coolers (cooled group [CL]; *n* = 4) or shaded barns without cooling (heat stressed group [HT]; *n* = 4) at the University of Florida Dairy Unit. All cows were cooled postpartum. Mammary biopsies were obtained at 14, 42, and 84 DIM. Proteomes were examined using iTRAQ technology at the UF Institute of Biotechnology Research by a Q Exactive Plus mass spectrometry system coupled to the NanoEasy nLC-1200. Data were searched against Uniprot Bos taurus database (45,234 contigs) using ProteinPilot v4.5. A meta-analysis from Student’s *t*-test was employed to test for protein expression differences between HT and CL at each time.

**Key Words:** heat stress, milk composition, milk protein expression, lactation biology, mammary proteome.
point. Proteins were differentially expressed (DEPs) at a fold change of less than 0.7 and greater than 1.3 and a $P$-value of < 0.05. A total of 4,964 proteins were identified. 251 unique proteins were differentially expressed between HT and CL. There were more upregulated proteins in HT compared with CL and the most DEPs occurred at 84 DIM. DEPs are involved in functions such as the heat shock response (e.g., ST13, HSPB1), energy metabolism (e.g., NDUFA4, ATP5O), carbohydrate metabolism (e.g., MDH1, TALDO1), amino acid metabolism (e.g., IVD, ASRGL1), fatty acid biosynthesis (e.g., ACSF3, ACACA), lactose synthesis (e.g., B4GALT1), and translation (e.g., RPS2, EIF1AX). These processes are critical for milk synthesis and the response to stress and may explain, at least in part, the impaired lactation performance of HT cows.

Key Words: proteomics, mammary gland, iTRAQ