Lactation Biology III

552 ASAS Centennial Presentation: Historical perspective on lactation biology. R. S. Kensinger*, *Oklahoma State University*, *Stillwater*.

Research over the past 100 years has revealed numerous exciting developments in our understanding of the biology of lactation. New assay technology combined with classical approaches to endocrinology contributed greatly to our knowledge of hormonal control of mammary function. The development of unconventional models of investigation (including suckling intensity, altered milking frequency, hemi-mastectomy, induced lactation, and transplantation of mammary glands) lead to greater understanding of factors that affect mammary function; as well as greater appreciation of the plasticity of the mammary gland. This era yielded papers on the characterization of the milk proteins and sequencing of the milk protein genes; elucidation of the complex array of milk lipids; and an appreciation for variation among species in oligosaccharides in milk. From a practical standpoint, this knowledge provided better management strategies to encourage passive transfer of immunity to neonates, and better selection tools for breeding females. Subtle benefits of colostrum and milk to neonates were described in recent years. The defense mechanisms of mammary glands were characterized in this era, and a greater appreciation of the impact of mastitis on livestock productivity was gained. This foundation knowledge lead to development of useful food animal practices and technologies like vaccination programs in pregnant females to enhance immunity in neonates, bST for dairy, and the development of Monensin for lactating cattle. This knowledge also encouraged the biomedical industry to use mammary glands in food animals to produce human pharmaceuticals.

Key Words: Centennial, Mammary, Lactation

553 ASAS Centennial Presentation: Lactation biology for the 21st century. J. J. Loor*[1] and W. Cohick[2], [1]*University of Illinois*, *Urbana*, [2]*Rutgers, The State University of New Jersey*, *New Brunswick*.

Knowledge of general aspects of mammary gland function, including metabolic pathways and hormonal regulation of mammary gland development and lactation, in livestock species was obtained several decades ago. As basic biological information of growth factor action, apoptotic mechanisms, and signal transduction events has exploded, the mouse became the model of choice for studying fundamental mechanisms regulating mammary function. A complete sequenced genome also has made the mouse amenable for studies of mammary gene network expression. Advances in molecular biology techniques currently allow researchers to genetically modify mice to either overexpress or completely lack specific genes, thereby studying their function in an in vivo setting. Furthermore, the use of mammary specific promoters has allowed genes related to mammary gland function to be eliminated from the mammary gland in a developmental and tissue-specific manner. These studies have provided compelling evidence of the underlying complexity that must ultimately allow the ruminant or swine mammary gland to function in a coordinated fashion throughout puberty, pregnancy, lactation, and involution. The challenge for the researcher interested in understanding these complex mechanisms to enhance the efficiency of milk production in domestic species is how to obtain similar information in much larger, expensive animals. One possible approach is to manipulate gene expression in vitro using mammary cell culture models derived from domestic animals, e.g. genes can be "knocked-down" using small interfering RNA approaches. Ultimately, major advances in understanding lactation biology may come from coupling basic mechanistic information with functional genomics, proteomics, and metabolomics approaches. A strong foundation in bioinformatics will also be required to optimize use of these new technologies. Additional areas that hold promise are stem cell biology and epigenetics. Strong training of our future scientists in these areas should facilitate livestock-focused mammary gland research that will allow basic information to be gained at unprecedented levels, ultimately leading to optimization of livestock production.

Key Words: Genomics, Gene Silencing, Stem Cells

554 The persistent milk yield response to frequent milking during early lactation is associated with persistent changes in mammary gene expression. E. H. Wall*, J. P. Bond, and T. B. McFadden, *University of Vermont*, *Burlington*.

Four-times daily milking during the first 3 weeks of lactation elicits an increase in milk yield, which persists through late lactation even after twice-daily milking is imposed. We hypothesized that this milk yield response would be associated with changes in mammary proliferation, apoptosis, and gene expression, which would persist throughout lactation. Six multiparous cows were assigned to unilateral frequent milking (**UFM**; twice daily milking of the left udder half (**2X**), four-times daily milking of the right udder half (**4X**)) on days 1 to 21 of lactation, followed by 2X thereafter. Udder halves initially milked 4X produced more milk than 2X glands during ($P < 0.001$), and after ($P < 0.05$) UFM treatment. To determine the mechanisms involved in the persistent milk yield response, we obtained mammary biopsies from both udder halves at 21, 23, and 40 days in milk (**DIM**). Rates of [^{3}H]-thymidine incorporation into DNA in vitro and mammary cell apoptosis were not affected by UFM or DIM ($P > 0.30$). Using Affymetrix GeneChip® Bovine Genome Arrays, we identified a cluster of 16 genes with a similar temporal pattern of expression that differed between 2× and 4× udder halves ($P < 0.05$). Nine of the genes in the cluster remained differentially expressed at 40 DIM ($P < 0.05$), indicating that they may be involved in the persistent milk yield response. Among these genes were chitinase-like protein (**CLP**)-1, clusterin, early growth response (**EGR**)-1, sex determining region Y-box (**SOX**)-4, and -9. These genes have been associated with mammary development, differentiation and remodeling; all of which may be functionally related to the increase in milk yield. We conclude that frequent milking during early lactation does not alter mammary growth but is associated with coordinated changes in mammary expression of 16 genes. Future experiments will determine the function of these genes in the mammary gland, and will clarify their role in the autocrine regulation of milk production and long-term alteration of mammary function.

Key Words: Frequent Milking, Gene Expression, Mammary Gland

555 Gene network analysis in mammary and liver tissue of lactating mice fed trans10,cis12-CLA. A. K. G. Kadegowda*[1], A. Thatcher[2], L. S. Piperova[1], S. L. Rodriguez-Zas[2], R. A. Erdman[1], and J. J. Loor[2], [1]*University of Maryland*, *College Park*, [2]*University of Illinois*, *Urbana*.

Exogenous trans10,cis12-CLA (10/12CLA) reduces mammary lipid synthesis in bovine and murine mammary tissue (MG). However, genome-wide alterations in MG and liver (LIV) associated with dietary 10/12CLA during lactation remain unknown. To better characterize MG and LIV tissue gene networks sensitive to 10/12CLA during lactation, we fed mice (n = 5/diet) control or control + 10/12CLA (37mg/day) between d 6 and d 10 post-partum. Tissues were harvested on d 10 after sacrifice. A 35,302 gene annotated murine exonic evidence-based oligo (MEEBO) microarray (70-mers) and quantitative PCR were used for transcript profiling. Cy3- and Cy5-labelled cDNA from MG or LIV and a reference standard were used for hybridizations (total of 20 microarrays). Milk fat concentration was 44% lower on d 10 vs. d 6 due to 10/12CLA. ANOVA (FDR-adjusted P = 0.20) identified 1,496 differentially expressed genes (DEG) in MG and 22 in LIV due to 10/12CLA. Among DEG in MG, we found 37 downregulated and 82 upregulated by 1.5-fold or greater. Gene network analysis of upregulated MG genes identified cell cycle (7 genes), cell growth/proliferation (10 genes), cell assembly/organization (27), and small molecule biochemistry (25) as modified families of related genes. Similar analysis of downregulated genes identified lipid metabolism (34 genes), cell death (29), and carbohydrate metabolism (23) as gene families most affected by 10/12CLA. The PPAR-signaling pathway was the most affected by 10/12CLA with a total of 77 DEG including Pparg and Pppard. Overall, results suggest that lower mammary lipid synthesis induced by dietary 10/12CLA in mice is associated with previously-unknown adaptations in gene networks in MG and to a lesser extent in LIV.

Key Words: Lactation, Genomics, t10c12 CLA