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SYMPOSIA AND ORAL SESSIONS
Triennial Lactation Symposium: Lactation Biology Training for the Next Generation—A Tribute to Dr. H. Allen Tucker

1 Bovine mammary epithelial cell lineages and parenchymal development. S. Ellis*[1], R. M. Akers[2], A. V. Capuco[3], and S. Safayi[1], *[1]Clemson University, Clemson, SC, [2]Virginia Polytechnic Institute, Blacksburg, VA, [3]USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD.*

Mammary development proceeds from an aggregation of cells in the ventral ectoderm to the establishment of an elaborate tree of alveoli, ducts, and cisternae. However, despite abundant data on endocrine regulation of ruminant mammary growth, we know comparatively little about cell lineages, expression of differentiation markers, and plasticity in mammary cell phenotype. Histologic analyses have revealed cell populations with distinct histochemical profiles, but functional assessment of the cell populations during development has been limited to analysis of proliferation and frequency estimations of morphotypes. The lack of transplantation models, limited availability of validated antibodies with reactivity to bovine antigens, and similar technical challenges have generally hindered the pace of discovery, but the application of new technologies like laser microdissection, transcriptional profiling, and multispectral image analysis are yielding important cues into bovine mammary cell ontogeny and developmental regulation. Our analyses have shown that prepubertal ovariectomy affects epithelial architecture, increases the proportion of cells expressing the estrogen receptor, and increases myoepithelial cell development, all concomitant with dramatic reduction in the mass of parenchymal tissue. Our observations point to a dual role for ovarian secretions in the control of not only the rate of epithelial development, but also the nature of the parenchymal development. The balanced stimulus and inhibition pathways are likely to cooperatively regulate mammary growth. The increased reliance on objective staining analyses and quantitative approaches will ensure broader repeatability, application, and extension of the findings. Advances in the understanding of mammary epithelial cell ontogeny, coupled with the established knowledge of endocrine factors affecting mammary development may yield intervention strategies to improve dairy profitability.

Key words: mammary development, cell lineage, prepubertal mammary growth

2 Prolactin—The multi-faceted potentiator of mammary growth and function. R. C. Hovey*, J. F. Trott, A. Schennink, W. K. Petrie, and M. K. VanKlompenberg, *University of California, Davis.*

Prolactin (PRL) confers numerous biological effects including its principle actions on the mammary glands during growth and lactation. These effects are mediated by dimers of the transmembrane PRL receptor (PRLR) and subsequent activation of numerous downstream signaling cascades. Traditional interpretations of how PRL acts on the mammary glands have generally built upon the concept that PRL acts directly, and independently, on the target epithelium during periods of mammary gland growth and lactation. In this presentation we will first review PRL action during mammary gland growth and lactation in several species with a focus on similarities and differences across PRLR and the control of its expression. We will then outline several lines of evidence to support the notion that many effects of PRL are mediated by additional mechanisms intrinsic to the mammary glands. Specifically, the effects of PRL on mammary gland growth are tightly coordinated by concomitant changes in the circulating levels of estrogen and progesterone, which in turn can modulate both PRLR expression and downstream gene expression. Furthermore, PRL not only acts on the epithelial population, but also modifies the stromal microenvironment by facilitating processes such as tissue remodeling and angiogenesis. Taken together, these modes of action highlight the many pathways by which PRL coordinately regulates and facilitates mammary gland growth during gestation before it initiates and regulates lactation.

Key words: prolactin, mammary gland, lactation

3 The lactocrine hypothesis: Programming reproductive tract development. F. F. Bartol*[1], J. C. Chen[2], D. J. Miller[1], A.-L. Frankshun[2], A. A. Wiley[1], A. J. Silva[1], M. E. Camp[2], K. M. Ferio[2], and C. A. Bagnell[2], *[1]Auburn University, Auburn, AL, [2]Rutgers University, New Brunswick, NJ.*

For eutherian mammals, a continuum of maternal support insures that development of progeny follows an optimal program. Beginning in utero, such support extends into the early neonatal period when nutrients and bioactive factors are communicated from mother to offspring in colostrum/milk. Defined as lactocrine signaling, communication of milk-borne bioactive factors (MbFs) from mother to offspring as a consequence of nursing is important for development of many somatic tissues, including the female reproductive tract. Endometrial development in the neonatal pig is estrogen receptor (ESR1) dependent. Disruption of ESR1-dependent, estrogen-sensitive developmental events shortly after birth (postnatal day = PND 0) that alter tissue developmental trajectory as determined on PND 14 can have long-term consequences for uterine function and reproductive capacity in adults. The lactocrine hypothesis for maternal programming of porcine endometrial development was proposed initially to explain how bioactive colostral relaxin (a porcine MbF), acting via its cognate receptor which is expressed in uterine tissues at birth, induces uterine expression of ESR1 and the parallel expression of other morphoregulatory factors such as vascular endothelial growth factor (VEGFA). This, it was proposed, insures propagation of down-stream signaling events essential to the success of endometrial development. To test the lactocrine hypothesis, patterns of uterine and endometrial gene expression and morphogenesis were evaluated at PND 2 and PND 14 in gilts that either nursed normally or were maintained in a lactocrine-null state on porcine milk replacer for defined periods from birth. Imposition of the lactocrine-null state for 48h from birth suppressed endometrial expression of morphoregulatory factors, including ESR1 and VEGFA, inhibited endometrial cell proliferation and retarded uterine gland development

by PND 14. Results provide the first evidence of cell compartment-specific, lactocrine-mediated endometrial morphogenesis and indicate that lactocrine signaling contributes to the palette of factors affecting reproductive tract development in the neonate.

Key words: lactocrine, uterus, development

4 Opportunities for improving milk production efficiency in dairy cattle. E. E. Connor*[1], J. L. Hutchison[2], K. M. Olson[2], and H. D. Norman[2], *[1]USDA-ARS, Bovine Functional Genomics Laboratory, Beltsville, MD, [2]USDA-ARS, Animal Improvement Programs Laboratory, Beltsville, MD.*

Increasing feed costs and the desire to improve environmental stewardship have stimulated interest in improving feed efficiency of livestock, including that of US dairy herds. For instance, USDA cost projections for corn and soybean meal suggest a 20% increase over 2010 pricing for a 16% protein mixed dairy cow ration in 2011, which may lead to a reduction in cow numbers to maintain profitability of dairy production. Furthermore, an October 2010 study by The Innovation Center for US Dairy to assess the carbon footprint of fluid milk found that the efficiency of feed conversion is the single greatest factor contributing to variation in the carbon footprint, due to its effects on methane release during enteric fermentation and from manure. Thus, we are conducting research to identify the most efficient dairy cattle at conversion of feed to milk using residual feed intake (RFI), a measure used successfully to identify the most efficient beef cattle at conversion of feed to gain. Residual feed intake is calculated as the difference between predicted and actual feed intake to support maintenance and production (e.g., growth in beef cattle, or milk in dairy cattle). Selection for a lower RFI phenotype can reduce feed intake, methane production, nutrient losses in manure, and visceral organ weights substantially in beef cattle. We have evaluated RFI measures during the first 90 d of lactation for the USDA-Beltsville Holstein herd and found the heritability of RFI to be 0.16 (n = 254). A difference in net feed intake of 8.3 kg/d DM was found between the least and most efficient animals. Mean actual DMI differed by 3.7 kg/d ($P < 0.0001$) between the efficient and inefficient groups (±0.5 SD from the mean RFI of 0), with no differences ($P > 0.20$) in mean BW, ADG, or ECM exhibited between the 2 groups. These results suggest promise for using RFI in dairy cattle to improve feed conversion to milk. Previous and current research on the use of RFI in lactating dairy cattle will be discussed, as well as opportunities to improve production efficiency of dairy cattle using RFI for milk production.

Key words: dairy cow, feed efficiency, residual feed intake

5 Lactational imprinting: The mechanism underlying the mammary response to changes in milking frequency? E. H. Wall*[1], J. P. Bond[2], and T. B. McFadden[3], *[1]Department of Animal Science, University of Vermont, Burlington, [2]Vermont Genetics Network Bioinformatics Core, University of Vermont, Burlington, [3]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.*

Regular removal of milk from the mammary gland is critical to maintaining milk secretion. Early studies in rodents demonstrated that changes in milking frequency influenced mammary cell number, DNA content and cell activity, and blood flow. Later studies in ruminants confirmed these observations, and confirmed that the response was regulated locally within the mammary gland. In addition, it was discovered that frequent milk removal during early lactation stimulated an increase in milk production that partially persisted through late lactation, indicating long-term effects on mammary function. The local mechanisms regulating the mammary response to changes in milking frequency are poorly understood, although several have been proposed. To gain insight into the mechanisms underlying the mammary response to changes in milking frequency, and to identify genes associated with the response, we used a functional genomics approach and conducted experiments on dairy cows exposed to unilateral frequent milking (UFM; twice daily milking (2X) of the left udder half, 4-times daily milking (4X) of the right udder half). Across multiple experiments, we were unable to detect an effect of UFM on mammary cell proliferation or apoptosis. We have identified, however, distinct transcriptional signatures associated with the mammary response to milk removal, milk stasis, and UFM during early lactation. Sequential sampling of mammary tissue revealed that when UFM was imposed during early lactation, a group of genes was coordinately regulated with changes in differential milk production. Moreover, some genes were persistently differentially expressed after UFM and were associated with the long-term increase in milk yield. We conclude that a coordinated transcriptional response underlies the increase in milk yield elicited by frequent milking during early lactation, and that the transcriptional signature may be a marker for the autocrine regulation of milk production. Moreover, we propose that we have identified a novel form of imprinting associated with long-term alteration of mammary function, which we term "lactational imprinting."

Key words: gene expression, imprinting, mammary gland

6 Mammary metabolism of amino acids in dairy cows. H. Lapierre*[1], L. Doepel[2], G. Raggio[3], and S. Lemosquet[4], *[1]Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, [2]University of Calgary, Calgary, AB, Canada, [3]College Alfred, Guelph University, Guelph, ON, Canada, [4]UMR1080 Dairy Production, INRA, Saint-Gilles, France.*

The mammary gland (MG) is the major net user of essential AA (EAA) supply. The MG metabolism of AA is not, however, a straightforward process. In the late 70s, 2 major groups of EAA were identified: Grp 1 [His, Met, Phe (+Tyr), Trp] for which MG uptake was similar to MP output, and Grp 2 (Ile, Leu, Val, Lys) for which uptake was greater than MP output. To confirm this and to expand our knowledge of MG metabolism, we have adapted and refined techniques including: measuring net flux with AA concentrations determined by isotopic dilution on individual rather than pooled samples, and measuring total flux using AA labeled with stable isotopes. Mammary net fluxes indicate that the stoichiometric transfer of Grp 1 AA is maintained under changing supply: the uptake to output ratio (U:O) is not different from 1. Using labeled Phe, we observed that doubling Phe supply did not result in any mammary oxidation of Phe + Tyr, indicating no alteration of the U:O. For Grp 2 AA, not only does the MG remove them in excess of MP secretion, but this excess increases with increasing supply. Using labeled Leu, we observed that Leu taken up in excess of MP secretion is oxidized within the MG, and that oxidation increased further with increased Leu excess induced by duodenal casein infusion. It is hypothesized that the excess uptake of Leu (and other Grp 2 AA) can provide ATP to support increased protein synthesis induced by increased protein supply or supply carbon skeleton for the synthesis of non-EAA or both. Using labeled Lys, we observed that under normal feeding conditions, Lys U:O averaged 1.37 with the N of Lys taken up in excess mainly transferred to Glx, Asx, Ser and Ala; with Lys depletion, lower Lys mammary excess (12%) was also transferred to other AA, but at lower rates. Arg is a unique EAA

as its U:O varies between 2 and 3. Deletion of Arg from an abomasal infusate decreased the U:O from 2.5 to 2.1 but did not alter MP output, suggesting that other sources of N can cover Arg deficiency. The MG metabolism of EAA is coordinated with splanchnic metabolism: the liver removes a large fraction of the portal absorption of Grp 1 AA whereas it removes, on a net basis, little if any Grp 2 AA.

Key words: dairy cow, amino acid, mammary

7 Stress effects on postpartum reproduction in dairy cows. M. A. Crowe* and E. J. Williams, *Veterinary Sciences Centre, University College Dublin, School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin 4, Ireland.*

The objective of the presentation is to review the effects of production stressors on reproductive performance of dairy cows. It has been well documented that genetic selection for milk yield over the last 50 years has been associated with reduced fertility. In addition to negative associations between yield and conception rate, there is also an association between milk production and expression of behavioral estrus. Stress caused by production diseases in high yielding dairy cows also contributes to the problems of poor fertility. Lameness results in reduced intensity of estrus, and can contribute to ovulation failure, which is largely due to reduced pre-ovulatory estradiol secretion and failure of the LH surge. Mastitis has been associated with prolonged intervals to dominant follicle selection, and in animals with uterine infection the dominant follicle grows slower and produces less estradiol. In a recent study we identified that milk yield was associated with an increased incidence of uterine infection, which is known to contribute to reduced fertility and prolonged calving to conception intervals. The incidence of uterine disease was 73% in high yielding, compared with 45% in low yielding cows. As well as effects at the ovary, various models of stress have also been shown to perturb endocrine secretion in the hypothalamus and anterior pituitary. In conclusion, the adverse affects on fertility associated with genetic selection for yield in dairy cows is in part associated with increased incidences of production disease induced stress but is also associated with high milk yield. Funded by SFI (07/SRC/B1156).

Key words: production disease, fertility, dairy cow