3 Management of photoperiod in the dairy herd for improved production and health. Geoffrey Dahl*[1] and Denis Petitclerc[2], [1]*University of Illinois,* [2]*AAFC-Dairy and Swine R&D Centre.*

Environmental influences on lactation efficiency are frequently associated with reductions in milk output. Heat stress, for example, leads to depressed feed intake and subsequently losses in production. Conversely, cold stress may limit nutrients available for milk synthesis. Fortunately, one environmental factor, photoperiod, can exert a positive effect on dairy performance when managed properly. Long days have consistently been shown to improve milk yield during established lactation. In addition, photoperiod management can be used to improve heifer growth and maximize accretion of lean tissue including mammary parenchyma. There is, however, evidence of refractoriness to long day stimulation. Recent work has focused on the dry period as a time when photoperiod manipulation can influence subsequent milk production. In contrast to lactating cows, multiparous cows benefit from exposure to short days when the dry period is followed by long days or ambient photoperiod after calving. Similarly, primiparous animals also respond positively to short days late in pregnancy when subsequently exposed to long days during lactation. Emerging evidence suggests that short days positively influence immune function in cattle. Mechanistically, it appears that prolactin has a causal relationship with the observed dairy performance effects during the dry period and on immune function, via altered sensitivity to prolactin through differential expression of prolactin receptor in multiple tissues. The objectives of this paper include a review of fundamental aspects of photoperiod physiology, integration of applied and basic research findings, and development of management recommendations for the entire life cycle of the dairy cow to optimize performance.

Key Words: Photoperiod, management, immune function

4 Effects of chronic oxytocin administration on oxytocin release and milk ejection efficiency. J. Macuhova[1], V. Tancin[1,2], and R. M. Bruckmaier[1], [1]*Institute of Physiology, Techn. Univ. Munich-Weihenstephan, Freising, Germany,* [2]*Research Institute of Animal Production, Nitra, Slovakia.*

The objective of this study was to test if reduced release of oxytocin (OT) from the pituitary or the sensitivity of OT receptors in the mammary gland are responsible for the reduced spontaneous milk ejection after long-term OT treatment. Fourteen healthy Brown Swiss dairy cows were used for the experiment. Cows were routinely milked twice daily at 5 a.m. and 4 p.m. in a 2x2 tandem milking parlour. They were randomly assigned to two treatment groups, seven animals in each group. During a period of 19 d they were i.m. injected with 5 ml NaCl solution (NaCl group) or 5 ml (50 IU) OT (OT group) 1 min before start of each milking. During evening milkings before and after chronic NaCl or OT treatment blood samples were collected at 1-min intervals for analysis of OT blood concentrations. At the end of these milkings OT (10 IU) was i.v. injected to remove residual milk. To detect changes in mammary gland sensitivity to OT, intramammary pressure (IMP) in the udder cistern was recorded during OT infusion before and after the chronic NaCl and OT treatment period. OT was infused at 0.15 IU/min, which caused a steady increase of OT blood concentration. The occurrence of milk ejection was visualized by an IMP rise in the cistern. Chronic NaCl treatment did not influence milk removal, OT release or IMP pattern. Chronic OT treatment reduced spontaneous milk removal by 15±5 %. OT release during milking was not reduced after chronic OT treatment. During OT infusion and IMP recording, commencement of milk ejection was similar before and after chronic OT treatment. However, time to reach IMP maximum was prolonged after chronic OT treatment ($p<0.05$). In conclusion, chronic OT administration did not change OT release nor OT blood concentration required to commence myoepithelial contraction. However, the intensity of myoepithelial contraction was reduced thus causing incomplete udder emptying.

Key Words: Oxytocin Treatment, Milk Ejection, Cow

5 Lactation persistency: insights from mammary cell proliferation studies. A.V. Capuco*[1], S.E. Ellis[2], S.A. Hale[3], E. Long[1], R.A. Erdman[3], X. Zhao[4], and M.J. Paape[1], [1]*USDA-ARS, Beltsville, MD,* [2]*Clemson University, Clemson, SC,* [3]*University of Maryland, College Park,* [4]*McGill University, Quebec, Canada.*

Milk yield is a function of the secretory activity and number of mammary epithelial cells. A persistent lactation is dependent upon maintaining number and activity of milk secreting cells with advancing lactation. When dairy cows are milked twice daily, the increase in milk yield from parturition to peak lactation is due to increased secretory activity per cell, rather than to accretion of additional epithelial cells. After peak lactation, declining milk yield is due to loss of mammary epithelial cells by apoptosis. During lactation, only 0.3% of mammary cells proliferate in a 24-h period. Yet this proliferative rate is sufficient to replace most mammary epithelial cells by the end of lactation. Management practices can influence lactation persistency. Administration of bovine somatotropin may enhance persistency by increasing cell proliferation and turnover, or by reducing the rate of apoptosis. Increased milking frequency during the first weeks of lactation increases milk yield even after return to less frequent milking, with increases of ∼10% over the entire lactation. A proliferative response to frequent milking during early lactation appears to be involved. Conversely, advanced pregnancy, infrequent milking, and mastitis increase death of epithelial cells by apoptosis. Regulation of mammary cell renewal provides a key to increasing persistency. Investigations to characterize epithelial cells that serve as the proliferative population in the bovine mammary gland have been initiated. Epithelial cells that stain lightly in histological sections are evident through all phases of mammary development and secretion, and account for nearly all proliferation in the prepubertal gland. Characterization of these cells may provide a means to regulate mammary cell proliferation and thus to enhance persistency, reduce the effects of mastitis, and decrease the necessity for a dry period.

6th Joint EAAP/ASAS Workshop on Biology of Lactation in Farm Animals
Lactation Biology in the Post-Genomic Era

6 Transgenic livestock: promise fulfilled. M.B. Wheeler*, *University of Illinois at Urbana-Champaign.*

Over the past two decades the ability to alter the genome of animals, by the introduction of DNA, has been a major technological advance in agriculture. Transgenic animals are produced by the introduction of a small, isolated, known fragment of DNA into pre-implantation embryos. This DNA is inserted into the chromosomes of the embryo and is expressed in all tissues of the resulting individual. The ability to move genes into organisms has been referred to as "gene transfer". This technique is of great importance to many aspects of biomedical science and agriculture. There are numerous potential applications of transgenic methodology to develop new or altered strains of agriculturally important livestock. Practical applications of transgenics in livestock production include improved milk production and composition, increased growth rate, improved feed utilization, improved carcass composition, increased disease resistance, enhanced reproductive performance, and increased prolificacy. The improvement of the nutrient or therapeutic value of milk may have a profound impact on survival and growth of newborns in both humans and animals. Transgenic pigs containing gene constructs (for the bovine milk protein alpha-lactalbumin) designed to improve sow milk have been produced. Results of these studies have shown the concentration of bovine alpha-lactalbumin was directly correlated with the concentration of endogenous porcine milk proteins throughout the 21 days of lactation. Milk production was higher in transgenic sows on days 3, 6 and 9 of lactation as compared to control sows. At weaning (d 21), piglets suckling the transgenic sows weighed 0.5 kg more than piglets suckling control sows. The use of transgenics to improve lactation can enhance offspring growth and may enhance offspring health in economically valuable livestock. The ultimate utility and value of transgenic technology will be limited by our ability to identify genes and appropriate regulatory sequences for the production of traits we wish to improve. Future improvements in nuclear transfer (cloning) technology, automation of embryo handling techniques and improvements in gene and/or chromosome transfer technology will in-

crease the efficiency of transgenic livestock production and allow more precise changes in the livestock genome.

Key Words: Lactation, Transgenic Animals, Swine

7 Transgenic models for animal science research and application. D.E. Kerr*[1], O. Wellnitz[1], A. Mitra[2], and R.J. Wall[2], [1]*University of Vermont,Burlington,* [2]*USDA-ARS, Beltsville, MD.*

Continual advances in the ability to produce transgenic animals make it likely that such animals will become important components of animal agriculture. Full benefit of the technology, and justification of its initial cost outlay, will be dependent on the establishment within these animals of new traits not easily achievable by other means. Potential applications include enhanced nutrient digestibility with reduced fecal losses, significantly altered milk composition, and enhanced disease resistance. Our goal is to enhance mastitis resistance of dairy cows by enabling the cells of the mammary gland to secrete addition antibacterial proteins. Proof of concept has been obtained through experimentation with a transgenic mouse model. Three lines of mice were developed that produce varying levels of lysostaphin in their milk. This protein has potent anti-staphylococcal activity and its secretion into milk confers substantial resistance to infection caused by intramammary challenge with *Staphylococcus aureus*, a major mastitis pathogen. Additional antibacterial proteins are being sought that will complement lysostaphin. A potential benefit of transgenic application of anti-bacterial proteins is the concomitant sparing in the agricultural use of anti-biotics currently used as human therapeutics. Anti-bacterial proteins are not typically used as injectable or oral therapeutics because of immune-mediated or digestive destruction of their activity. In contrast, the immune system of transgenic animals will not consider the transgenic protein as being foreign. In addition we are exploring the potential of involution or mastitis responsive promoter elements for use in subsequent transgenic experiments designed to restrict lysostaphin production to these important time points. It is anticipated that genomics will play a role is unveiling additional genes whose promoter elements will enable desired temporal expression patterns. The transgenic approach to insertion of new genetic material into agriculturally important animals is feasible but requires extensive prior evaluation of the transgene and transgene product in model systems.

Key Words: Mastitis

8 Regulation of apoptosis in mammary gland of cows at early lactation. M. Colitti* and B. Stefanon, *Dipartimento di Scienze della Produzione Animale - Universita' di Udine, Italy.*

Apoptosis inducing factor (AIF) and bcl-2 proteins are involved in apoptosis control, but little is known about their interaction in lactation of cattle. In the present paper the onset of apoptosis and apoptosis-related signals in mammary gland at the beginning of lactation have been investigated . In addition a partial complementary DNA (cDNA) for bovine AIF has been identified and its expression evaluated. Mammary gland tissue was collected from 3 first-calving cows by biopsy at early lactation. The samples were processed for total RNA extraction and RT-PCR analysis were performed for bcl-2, bax, bcl-X and AIF genes. For AIF, ClustalX software was also utilised to align the coding sequences (cds) for rat (Genebank, accession AB04723), human (Genebank, accession XM010246) and mouse (Genebank, accession BC003292) AIF. Highly conserved regions of the AIF cds between the examined species were assessed with Genedoc software. Amplification and sequencing of AIF cDNA from bovine mammary tissue revealed a high degree of homology. In particular, the bovine AIF partial-cd was highly homologous (89%) between nucleotides 1584-1786 of the rat AIF sequence and nucleotides 1541-1743 that encode for the human PDCD8 (91%). The amino acid sequence of bovine AIF showed still higher similarity between species, with 96% homology for rat AIF (residues 496-562) and 93% with that of the human protein (residues 501-567). Within the time course of this experiment, we found a steady-state of bcl-2 and bcl-x expression and the up and down regulation of bax RNAs, which could indicate that in lactating cows these genes and related proteins are differently involved in apoptosis compare to mice. The *in situ* hybridisation data showed that the epithelial cells contained AIF expressed at a intracytoplasmatic level, but not into the nucleus. It was demonstrated that no AIF translocation was detectable in bcl-2 overexpressing cells and this could suggest that in mammary tissue during early lactation the protein was confined to the mitochondrial intermembrane space, in agreement with the low apoptotic index observed.

Key Words: Apoptosis Inducing Factor, Mammary gland, Dairy cows

9 Proliferation-associated gene expression in bovine mammary gland. T. B. McFadden*, *University of Vermont.*

Mammary development is a crucial determinant of potential milk producing capacity in dairy cows. Fundamentally, milk production is a function of the number and synthetic activity of secretory cells in the udder. Optimal nutrition and management allow for full expression of lactational potential. Therefore, manipulation of mammary growth in developing heifers and dry cows offers an opportunity to increase the efficiency of milk production. However, realization of this opportunity will require substantial increases in understanding of the basic mechanisms that regulate mammary development. Currently, a wide variety of factors are known to influence mammary growth, including genetic merit, nutritional management, hormonal regulation, physiological state and photoperiod. Unfortunately, relatively little detail on underlying mechanisms is available. In recent years, rapid advances in genomic technology have made it possible to conduct high-throughput screening of tens of thousands of genes in an effort to determine relationships between levels of gene expression and physiological function. Such "functional genomics" experiments yield gene expression profiles that may confirm known roles of particular genes while illuminating associations with novel genes, or previously unsuspected involvement of known genes. Using such an approach, we recently identified 200 candidate genes whose levels of mRNA expression were strongly associated with proliferation of mammary cells. Ongoing studies with a subset of these genes are aimed at confirming their relevance and further characterizing the regulation of their expression and their roles in control of mammary development. The objectives of this paper are to provide an overview of the factors that influence mammary development, to discuss fundamental concepts underlying genomic approaches, and to illustrate application of these techniques to studying regulation of mammary development and potential applications.

Key Words: Proliferation, Gene Expression, Mammary Development

10 Molecular methods for probing signal transduction pathways in mammary tissue. L.G. Sheffield*, *University of Wisconsin, Madison.*

Expression profiling studies indicate that expression of a large number of genes is altered during lactation. Among these are members of the amphiregulin family, including epidermal growth factor (EGF). Lactating mammary tissue expresses 5-10 times as much EGF as tissue from pregnant or otherwise nonlactating animals, with almost all of the expression localized to secretory epithelial cells. Although much of the EGF is processed for secretion, some appears to remain as a partly processed 40-45 kDa transmembrane protein that includes the EGF domain as well as several EGF-like repeats. One potential ligand for this transmembrane protein is a soluble form of the EGF receptor, consisting predominantly of the extracellular domain of the receptor. EGF receptor extracellular domain induces tyrosine phosphorylation of cytoskeletal proteins in cells that express EGF, but not in cells lacking EGF expression. Although the intracellular domain of EGF lacks any kinase activity, it appears to physically associate with at least one as yet unidentified protein kinase. Activity of the EGF-associated kinase is increased by treatment with the extracellular domain of EGF receptor, apparently independently of transmembrane EGF receptor. Our laboratory is currently using a variety of proteomics approaches to identify proteins that interact with the membrane bound forms of EGF. Recombinantly produced intracellular domain of EGF fused with a 5X histidine tag is used as a bait protein in co-precipitation assays. Similarly, co-immunoprecipitation of EGF is used to verify results. These methods, when combined with microsequencing and/or matrix-assisted laser desorption mass spectrometry (MALDI MS) can identify interactions with known proteins. Yeast two hybrid approaches are used to identify interactions with unidentified proteins. In addition, DNA array approaches are being used to explore the possible pathways by which transmembrane forms of EGF can modify cell physiology. Results to date suggest that transmembrane forms of EGF may have a role in limiting cell proliferation or activating tumor suppressor pathways. Similar techniques are applicable to a variety of other signaling systems.

Key Words: Genomics, Proteomics, Mammary Development