radically modified to respond to the challenges of open economies and international trade. During the last decade these policies have been directed towards providing direct support to production, to enhance trade and to promote the development and transfer of technology. Presently, Mexico has signed several free trade agreements with NAFTA being the most notable one. Animal production has shown remarkable annual growth (4.9% for meat, 4.3% for dairy and 6.3% for egg) during the last 10 years. Due to increased demand and trade liberalization, the net trade balance for the sector has been negative, accounted primarily by a deficit in dairy and meat, a condition that is also observed in processed animal products, where dairy cause more than 50% of this deficit. In contrast with the importance of the rural sector, during the last decade public spending has not only been reduced but diversified among programs and institutions with many contradictory goals. Among challenges for the future are the need to increase production, productivity and sustainability of the livestock sector, the development of new products for a more demanding population, improvement of animal welfare and protection and recovery of natural resources. These challenges require animal scientist willing to find new paradigms and to participate in activities traditionally considered outside the scope of animal science.

Key Words: Agricultural policies, Trade agreements, Animal science

Milk Synthesis
Regulation of Mammary Gland Function by Growth Factors and Downstream Signaling Cascades

204 Effect of transforming growth factor-beta-1 on mammary development.

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The mammary gland is dependent on hormones and growth factors for development and differentiation. While studies have been conducted to understand the role of growth factors that stimulate mammary growth, few studies have focused on the role of growth inhibitors in mammary development. Transforming growth factor - beta 1 (TGF-β1) is a potent inhibitor of mammary growth and stimulates the extracellular matrix (ECM). The ECM effects mammary development because it provides the scaffolding that supports the epithelial cells of the gland. We studied the effect of TGF-β on mammary development in cell lines and mammary tissue. Incubation of mammary epithelial cells with 2.5 - 5 ng/ml of TGF-β1 + 10% fetal bovine serum (FBS) results in at least a 50% reduction in cell growth compared to FBS controls. Using flow cytometry, we characterized changes in the cell cycle in response to TGF-β. NOG-8 cells were synchronized by serum starvation for 48 hours followed by incubation in media supplemented with 10% FBS and 0 or 2.5 ng/ml TGF-β1. Cells were harvested at 6, 48, and 72 hours post-treatment. There were fewer cells in G0/G1 and a higher proportion of dying cells in the TGF-β treated cells compared to controls at all time points. A cell cycle specific mini-array is being used to determine which genes in the cell cycle are changed in response to TGF-β1. In addition to the effects on cell cycle, TGF-β also effects the production of extracellular matrix proteins, which causes a change in cell shape and function. Cells exposed to 5 ng/ml TGF-β1 changed from a cobblestone morphology to elongated fibroblast-like morphology and expressed high levels of fibronectin as determined by immunocytochemistry. The cells continued to be growth inhibited. Last, studies are underway to determine the in vivo effects of TGF-β on the mammary gland of heifers. Changes in DNA synthesis, cell cycle gene expression and expression of extracellular matrix proteins are being used to measure the response. These studies will lead to a better understanding of how TGF-β effects mammary cell growth.

Key Words: mammary, transforming growth factor beta, growth inhibitor

205 Mammary development, growth and plasma levels of IGF-I and IGF-binding proteins in gilts provided different energy levels from weaning to puberty.

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We investigated the effect of feeding level from weaning (d 28) to slaughter at puberty (d 162) on growth rate, mammary development and plasma levels of IGF-I and IGF-binding proteins (IGFBP) in 10 litters of 4 female pigs. From d 28 to 90 (period 1) and from d 90 to 162 (period 2), pigs were fed either ad libitum (A) or restrictively (R; i.e. 30% lower feed intake in period 1 and 25% lower in period 2) in a 2x2 factorial design with treatments named AA, AR, RA and RR. In period 1, ADG of A-gilts was 622 g vs. 522 g for R-gilts (P<0.001). At the end of period 1, A- compared with R-gilts had higher plasma levels of IGF-I (303 vs. 220 ng/ml, P<0.01) and IGFBP-3 (770 vs. 564, arbitrary units, P<0.01), but lower IGFBP-2 (291 vs. 396 a.u., P<0.02) and 28 kDa IGFBP (P<0.06). In period 2, ADG of RA- and AA-gilts was 1012 g vs. 792 g for RR- and AR-gilts (P<0.001). Furthermore, RA-gilts showed compensatory growth compared with AA-gilts (1054 vs. 971 g/d, P<0.07) with no difference in feed intake. At the end of period 2, there was a tendency for higher plasma IGF-I (P<0.15) in AA- and RA-gilts compared with AR- and RR-gilts whereas IGFBP-2 and 28 kDa IGFBP were reduced (P<0.01). The amount of dissected mammary tissue was higher in AA- and RA-gilts compared with AR- and RR-gilts (86 vs. 59 g/gland, P<0.001), and although DNA concentration was lower in AA- and RA-gilts compared with AR- and RR-gilts (342 vs. 397 μg/g tissue, P<0.04), total amount of mammary DNA was highest in AA- and RA-gilts. The concentration of mammary RNA was not affected by treatment. Feeding level in period 1 did not affect the mammary measures. We conclude that to obtain high mammary growth, a period with ad libitum feeding before puberty is needed, however, this period does not have to commence at weaning. Furthermore, differences in growth rate are associated with differences in IGF-I and IGFBPs, and female pigs fed restrictively from weaning to d 90 and ad libitum until puberty grow as fast as do continuously ad libitum fed pigs.

Key Words: mammary, gilt, IGF

206 Polycation-mediated transfection of the porcine mammary gland.

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Production of recombinant proteins in the milk of livestock has thus far been limited to transgenic and viral-mediated gene transfer methods. Our previous studies have demonstrated the feasibility of polycation-mediated transfection of bovine and murine mammary cells *in vitro* and the guinea pig mammary gland *in vivo*. These experiments were conducted to determine if direct intramammary infusion of polycation-DNA complexes in the porcine mammary gland would result in recombinant human growth hormone (hGH) secretion in milk. A second parity Yorkshire sow (sow 1) was tranquilized on day 112 of gestation. Teat ends were cleaned with alcohol and mammary glands transfected by infusing each teat opening with 50 ml of HBSS containing either; DEAE-dextran (DEAE) 1.25 mg/ml (sham transfection), or DEAE-dextran 1.25 mg/ml and plasmid DNA (50 ug/ml). Following parturition milk samples were collected daily, defatted, and stored at -80°C until assayed for hGH by radioimmunoassay. Milk from sham transfected and uninfused mammary glands contained no hGH. Milk from the DEAE-DNA transfected mammary gland contained hGH on all 14 days of lactation with expression peaking on day 6 at 5.5 ng hGH/ml and declining to 1.5 ng/ml by day 14. A second experiment was conducted as described above utilizing a first litter gilt (sow 2). Milk from two mammary glands transfected with DEAE-DNA again contained hGH throughout the first 12 days of lactation while sham and control samples contained none. Expression profiles for sow 2 were similar to sow 1 with expression peaking at 1.1 (gland 3) and 0.88 (gland 2) ng hGH/ml on days 8 and 9 respectively. Differences in hGH expression levels may be due to variation in time from transfection to parturition (3 days for sow 1 vs. 6 days for sow 2) or parity differences. Although expression levels differ between animals these results demonstrate the feasibility of transfecting the porcine mammary gland via direct intramammary infusion.

Key Words: Transfection, Porcine, Polycation

207 Frequent milking in early lactation that increases milk yield also increases prolactin receptor mRNA expression. G. Dahl*, T. Auchtung, J. Underwood, and J. Drackley, *University of Illinois.*

The periparturient surge of prolactin (PRL) is essential to optimize lactogenesis in cattle. The importance of PRL following parturition in cattle, however, has previously been thought to be limited. Frequent milking early in lactation (i.e., pre-peak) causes persistent increases in milk yield for that lactation, yet the mechanism for that response is unknown. This experiment tested the hypothesis that more frequent milking increases PRL sensitivity early in lactation and subsequently leads to higher milk yield. Jersey cows (n=4/treatment) were milked twice (2X) or four times (4X) each day for the first 21 d of lactation and subsequently all were milked 2X. Blood samples were collected on d 4, 7, 14, and 21 for quantification of PRL in plasma. Additionally, lymphocytes were harvested and prolactin receptor (long and short forms; PRL-R) mRNA was quantified as a proxy for PRL-R expression in mammary epithelial cells. Relative to 2X, 4X increased yield during the first 21 d of lactation from 21.8 to 26.8 kg/d (SED = 2.8 kg/d; P<0.05), and the difference persisted for at least the next 21 d (2X = 27.3 vs. 4X = 33.1 kg/d; SED = 2.7; P<0.056). Compared with 2X, 4X increased the concentration of PRL in plasma from 8.3 to 10.1 ng/mL (SED = 1.3; P<0.11). Expression of mRNA for short and long forms of PRL-R was greater in 4X cows on d 4 relative to 2X cows (P<0.02). In summary, doubling the frequency of milking for the first 21 d of lactation caused a persistent increase in milk yield, greater PRL in plasma and increased PRL-R mRNA expression. We conclude that more frequent milking early in lactation increases PRL sensitivity in the mammary gland through greater release and reception of PRL signaling.

Key Words: prolactin, receptor, frequent milking

208 Effect of growth factors and hormones on mammogenesis and lactogenesis in cattle. Robert Collier*[1], J.C. Byatt[2], M.F. McGrath[2], P.J. Eppard[2], J.L. Vicini[2], and C. Stiening[1], [1]*University of Arizona, Department of Animal Sciences,* [2]*Monsanto Company.*

In vitro and in vivo models were employed to test effects of growth factors and hormones on mammogenesis in cattle. Bovine mammary collagen gel culture was utilized to identify direct acting mitogens. These were then infused via the streak canal in half udder studies utilizing pregnant dairy and beef cattle to determine if mammogenic activity identified in vitro was present in vivo. Hormones and growth factors identified as mammogenic in vivo were then utilized in half and full udder lactation trials to determine if increased mammogenesis translated into increased milk yield. Growth factors identified as mammogenic in vitro and in vivo were insulin-like growth factor I, epidermal growth factor and transforming growth factor alpha. Hormones identified as mammogenic in vivo were prostaglandins of the E series, bovine growth hormone and bovine placental lactogen. All of these were tested in half and full udder lactation trials by intramammary infusion in the last trimester of pregnancy with exception of bovine placental lactogen which was administered systemically. All failed to demonstrate an increase in milk production post-partum. Prostaglandins of the E series (PGE) were shown to cause large increases in udder volume and secretory activity when infused in the mammary gland during late pregnancy. Further studies demonstrated that PGE is synthesized by mammary tissue and concentration of PGE in mammary secretion increases dramatically during the peripartum period. It is proposed that PGE is involved in Stage II lactogenesis. At present, we cannot identify a mammogenic hormone treatment which results in increased milk yield postpartum. This may indicate that lactation potential is established during ductal development prior to onset of pregnancy.

Key Words: hormones, growth factors, mammogenesis

209 Both phosphatidylinositol 3-kinase (PI3K) and mitogen activated protein kinase (MAPK) pathways are required for IGF-I regulation of IGF binding protein-5 synthesis in bovine mammary cells. J. Fleming* and W. Cohick, *Rutgers University.*

IGF-I is an important mediator of mammary epithelial cell (MEC) proliferation, and thus is critical to the normal development of the mammary gland. The IGF binding proteins (IGFBP) modulate cell growth through both IGF-dependent and independent mechanisms. Since the development of the mammary epithelium involves complex interactions between stromal and secretory components, we investigated IGFBP synthesis by primary bovine mammary fibroblasts (BMF). BMF were found to synthesize IGFBP-2, -3, and -5 by Northern analysis. IGFBP-5 mRNA levels were increased 4.5-fold over serum-free controls by IGF-I treatment (200 ng/ml) for 8 hr. Increases in IGFBP-3 mRNA levels were also observed, though to a lesser degree. In contrast, IGFBP-2 mRNA levels were unchanged. To determine if the effect of IGF-I on IGFBP-5 mRNA levels was specific to this cell type, we investigated the effect in the MEC line MAC-T. IGFBP-5 mRNA levels were similarly increased by IGF-I in MAC-T cells. To determine the intracellular pathways used by IGF-I to regulate IGFBP-5 synthesis, we first examined which pathways are activated by IGF-I. Western analysis of cell lysates using antibodies specific to phosphorylated forms of the proteins revealed that IGF-I activated components of both the PI3K and the MAPK cascades in BMF. Activation of both AKT and ERK 1/2 by IGF-I was observed at 5 min and was maximal between 15 and 30 min. We have previously reported that IGF-I stimulates the PI3K, but not the MAPK pathway in MAC-T cells. To determine which of these pathways mediates IGF-I stimulation of IGFBP-5, inhibitors of the PI3K (LY294002) and MAPK (PD98059) pathways were employed. In both BMF and MAC-T cells, inhibition of either pathway alone decreased IGFBP-5 synthesis by at least 50%. When both pathways were concurrently blocked, an even larger decrease in synthesis was observed. These studies indicate that while IGF-I activates different signaling pathways downstream of its receptor in BMF compared to MAC-T cells, both the PI3K and the MAPK pathways are required for IGF-I stimulated synthesis of IGFBP-5.

Key Words: Insulin-like growth factor-I, IGF binding protein, Mammary

210 Parathyroid hormone-related peptide (PTHrP) enhances mammary tight junction (TJ) formation under low-calcium (Ca) conditions through maintaining intracellular Ca stores. K. Stelwagen* and M. R. Callaghan, *AgResearch Ltd., Hamilton, New Zealand.*

TJ play an essential role in cell-cell contact between epithelial cells and as such play a critical role in cell functioning. We have previously demonstrated the requirements of endocrine factors (glucocorticoids, prolactin) and extracellular Ca for the formation and maintenance of mammary TJ. Given that PTHrP is involved in cellular Ca homeostasis, we postulated a role for PTHrP in the regulation of mammary TJ. The effect of PTHrP on TJ was studied in the mouse mammary cell line COMMA-1D by measuring transepithelial electrical resistance (TER) across the cell monolayer. Data shown are after 24 h culture in low-Ca (2 μM) medium. PTHrP (0, 10 or 100 nM) did not affect TER in cells in normal-Ca (1.8 mM) medium. However, when cells were kept in a low-Ca medium PTHrP increased TER in a dose-related fashion (PTHrP, 0 nM *vs.* 10 nM *vs.* 100 nM: 544^a *vs.* 563^a *vs.* $686^b \pm 42$ $\Omega.cm^2$, ^{ab}P<0.05). The presence of an apical Ca-channel activator (Bay K-8644, 50 μM) increased the TER beyond that of PTHrP alone (0 nM *vs.* 100 nM *vs.* 100 nM+Bay K *vs.* Bay K alone: 896^a *vs.* 1036^{ab} *vs.* 1198^b *vs.* $907^a \pm 81$ $\Omega.cm^2$, ^{ab}P<0.05), whereas PTHrP did not affect TER in the presence of a Ca-channel blocker (Nifedipine, 50 μM; 0 nM *vs.* 100 nM *vs.* 100 nM+Nifedipine *vs.* Nifedipine alone: 1250^a *vs.* 1604^b *vs.* 438^c *vs.* $405^c \pm 80$ $\Omega.cm^2$, ^{abc}P<0.05). Western analyses showed that the expression of the major TJ protein occludin was highest with 0 nM PTHrP and lowest with the 100 nM dose, approaching that observed in cells grown in normal-Ca medium. This indicates that with a low-Ca challenge there is enhanced TJ synthesis (repair), but that there is less synthesis occurring as the level of PTHrP increases, which increasingly facilitates a replenishment and/or maintenance of intracellular Ca stores. These data corroborate the TER data. In conclusion, PTHrP enhances mammary TJ formation when extracellular Ca is limiting by maintaining intracellular Ca supplies.

Key Words: Mammary, Tight junction, PTHrP