

Physiology and Endocrinology: The Physiology of Gestation and the Post-partum Interval

470 Fibroblast growth factor 2-induced expression of interferon-tau is mediated by protein kinase C in bovine trophectoderm. Q. Yang*, S. E. Johnson, and A. D. Ealy, *University of Florida, Gainesville*.

Interferon-tau (IFNT) serves as the maternal recognition of pregnancy signal in cattle and sheep. Pre- and peri-attachment conceptus production of IFNT is controlled in part by members of the fibroblast growth factor (FGF) family. FGF2 supplementation stimulates IFNT mRNA and protein concentrations in bovine blastocysts and trophectoderm. The objective of this work was to identify the intracellular signaling modules invoked by FGF2 in bovine trophectoderm cells (CT1). CT1 cells were treated for 2 h with chemical inhibitors for either protein kinase C (PKC) isoforms (0.5 μ M Calphostin), extracellular signal-regulated kinases (ERK)1/2 (50 μ M PD98059), p38 mitogen-activated protein kinase (MAPK) (25 μ M SB203580) or vehicle-only prior to FGF2 supplementation for 24 h. After cell lysis and RNA extraction, quantitative RT-PCR revealed a 10.70 ± 1.93 fold increase ($P=0.001$) in IFNT mRNA abundance in response to FGF2. Suppression of ERK1/2 or p38 activity did not alter the FGF2 response. However, exposure to the pan-PKC inhibitor limited ($P=0.01$) the ability of FGF2 to increase in IFNT mRNA abundance (2.42 ± 1.13 fold induction above control). Media supplementation with 100 nM phorbol 12-myristate 13-acetate (PMA), a PKC activator, mimicked the FGF2 response in CT1 cells. Selectivity of the PKC response was examined in the presence of chemical antagonists to PKC- $\alpha/\beta/\gamma$ (5 μ M Gö6976) or PKC- δ/θ (5 μ M rottlerin). Treatment of CT1 cells with Gö6976 did not affect FGF2- or PMA-mediated increases in IFNT mRNA abundance. By contrast, inhibition of PKC- δ/θ attenuated ($P<0.01$) the PMA and FGF2 responses. Thus, PKC- δ or - θ likely is required for FGF2-mediated IFNT expression. Further studies are forthcoming to confirm this and other roles for PKC- δ/θ during bovine conceptus development. Project supported by National Research Initiative Competitive Grant #2003-35203-13345 and 2003-35203-15382 from the USDA Cooperative State Research, Education, and Extension Service.

Key Words: Pregnancy, Placenta, Gene Expression

471 Expression of fibroblast growth factors and their receptors during pre- and peri-attachment development in bovine conceptuses. F. N. T. Cooke* and A. D. Ealy, *The University of Florida, Gainesville*.

For pregnancy to continue beyond the length of a normal estrous cycle, the bovine conceptus must secrete copious quantities of interferon-tau (IFNT) to interrupt the luteolytic process and maintain pregnancy. This laboratory discovered that fibroblast growth factor 2 (FGF2) increases IFNT production in cultured bovine trophectoderm and blastocysts. Several genes encode FGFs and some of these FGFs possess receptor subtype binding affinities similar to FGF2. To better understand the functional roles of FGFs during bovine conceptus development, studies were completed to: 1) identify the FGF receptor (FGFR) subtypes expressed by pre- and peri-attachment bovine conceptuses, and 2) identify conceptus-derived FGFs that may interact with these receptors. RT-PCR was completed using total RNA extracted from in vitro-derived bovine

blastocysts and in vivo-derived elongated conceptuses (3 pools/stage) and primers specific for bovine FGFR1, 2, 3 or 4. Sequencing of the amplified products revealed expression of receptor subtypes FGFR1IIIc, 2IIIb, 3IIIc, and 4 at both stages of development. Since FGFR2IIIb is crucial for trophectoderm development in other species, expression of ligands that act through this receptor subtype was explored. Transcripts for FGF1, 2, and 10 but not FGF7 were detected in elongated bovine conceptuses. In blastocysts, FGF1 and 2 mRNA abundance was low and FGF10 transcripts were not amplified. FGF7 transcript abundance was not analyzed at the blastocysts stage. In summary, at least four FGFRs reside in pre- and peri-attachment bovine conceptuses. Moreover, conceptuses express at least three candidate FGFs during elongation, the time of peak IFNT expression. Further studies are required to examine how some or all of these ligands interact with their receptors to regulate IFNT expression and conceptus development in cattle and other ruminants. Project supported by National Research Initiative Competitive Grant #2003-35203-13345 and 2003-35203-15382 from the USDA Cooperative State Research, Education, and Extension Service.

Key Words: Embryo, Pregnancy, Placenta

472 Reduced angiogenic factor expression in cotyledonary (COT) arteries of overnourished, obese ewes at midgestation. Y. Ma*¹, M. J. Zhu¹, P. W. Nathanielsz^{1,2}, and S. P. Ford¹, ¹*University of Wyoming, Laramie*, ²*University of Texas, San Antonio*.

In the sheep, maternal:fetal exchange takes place in placentomes, which are comprised of maternal caruncular and fetal COT tissues. We have demonstrated previously that COT vascularity in obese ewes was decreased ~50% by 75 d gestational age (dGA). This study investigated the role of selected angiogenic factors in reducing COT vascularity in pregnant obese ewes. Multiparous ewes carrying twin fetuses were assigned to a control (C, 100% of NRC recommendations; n = 10) or obesogenic (OB, 150% of NRC; n = 10) diet from 60 d before conception to 75 dGA, at which time COT arteries were collected from 5 ewes/group and snap frozen on liquid nitrogen. The remaining ewes in each group were maintained on their respective diets and allowed to lamb. At 75 dGA, OB ewes and their fetuses were ~50% and ~25% heavier, respectively, than C ewes and fetuses. Lambs from both dietary groups exhibited similar birth weights. COT artery mRNA levels of vascular endothelial growth factor (VEGF) and its receptors FLT-1 and KDR, angiopoietin-1 (ANG-1) and -2 (ANG-2), and their receptor Tie-2, basic fibroblast growth factor (FGF-2), and placenta growth factor (PLGF) were quantified via Real-time PCR. VEGF, ANG-1, ANG-2, FGF-2 and PLGF mRNA level were reduced ($P<0.05$) 2 fold or more in COT arteries of OB vs. C ewes, while no treatment differences were observed in FLT-1, KDR, or Tie-2 expression. COT arterial protein levels of VEGF, PLGF and FGF-2 were then quantified by Western-blot, and were reduced ($P<0.05$) 41%, 32% and 50%, respectively in OB ewes when compared to C ewes. This decrease in COT artery angiogenic factor expression in OB ewes would be expected to decrease vascularity and thus nutrient delivery to the fetus, slowing fetal growth, and reducing lamb birth weight. NIH INBRE 1P20RR16474.

Key Words: Angiogenic Factors, COT Vascularity, Obese Ewe

473 Increased circulating progesterone (P₄) levels during the estrous cycle in offspring of nutrient restricted ewes. L. A. George*¹, P. W. Nathanielsz^{1,2}, and S. P. Ford¹, ¹University of Wyoming, Laramie, ²University of Texas, San Antonio.

We have reported that fetuses of ewes nutrient restricted from 28-78 d of gestation (dGA) exhibited increased oxidative base lesions within DNA of day 78 fetal oogonia compared to controls (Murdoch et al., 2003, *Reprod. Biol. Endocrinol.* 1:6). Such lesions in fetal oogonia could affect oocyte/follicular/corpora lutea (CL) function later in life. P₄ secretion by the CL is vital for initiation and maintenance of cyclicity and pregnancy. This study examined the impact of maternal nutrient restriction during early gestation on P₄ secretion during an estrous cycle of female offspring. Dams were fed either a nutrient restricted (NR: 50% NRC recommendations) or control (C: 100% NRC) diet from 28 to 78 dGA and then both groups were fed 100% of requirements to lambing. Female lambs from NR and C dams were commingled in a single group and fed 100% NRC from weaning through testing. During October and November, yearlings born from NR (n=7) and C (n=7) dams were monitored daily for estrus and blood sampled every three days from standing estrus (d 0) to the subsequent estrus. This procedure was repeated at 3 yrs of age. NR and C ewes were of similar BW both at 1 (62.4 ± 3.1 kg) and 3 (75.8 ± 2.6 kg) yrs of age. Estrous cycle lengths were similar across treatment groups (19.1 ± 0.2 d). P₄ area under the curve was 20% higher in NR vs. C offspring at 1 yr of age ($P = 0.05$), and 23% higher at 3 yrs of age ($P = 0.08$). Maternal diet-induced increases in systemic P₄ in female offspring of NR vs. C ewes could indicate differences in either CL P₄ secretion, incidence of multiple ovulations and/or P₄ clearance. Regardless of mechanism, these data provide evidence for the persistent in utero programming of progesterone levels in offspring induced by differences in early gestational maternal nutrition.

Key Words: Fetal Programming, Estrous Cycle, Progesterone

474 Increased macrophage migration inhibitory factor (MIF) in the pancreas of fetuses gestated by overnourished, obese ewes. L. Zhang*¹, M. J. Zhu¹, P. W. Nathanielsz^{1,2}, and S. P. Ford¹, ¹University of Wyoming, Laramie, ²University of Texas Health Sciences Center, San Antonio, TX.

MIF is an integral component of the stress response and has been shown to be released by the pituitary, macrophage, and the T-lymphocyte in response to inflammation, infection and stress. More recently MIF was identified as a glucose-dependent pancreatic beta cell product, which increases insulin secretion in an autocrine fashion, and thus may play an important role in carbohydrate metabolism (Waeber et al. 1997. *Proc. Natl. Acad. Sci.* 94:4782-4787). In a recently developed sheep model of maternal obesity, we have reported that blood glucose and insulin concentrations were markedly elevated at midgestation in fetuses from obese mothers. This study investigated the potential role of MIF in the regulation of fetal pancreatic insulin secretion in our obese model. Multiparous ewes carrying twin fetuses were assigned to a control (C, 100% of NRC recommendations; n=5) or obesogenic (OB, 150% of NRC; n=5) diet from 60 d before conception to 75 days of gestation (dGA), at which time fetal pancreatic tissue were collected and snap frozen on liquid nitrogen or fixed in paraformaldehyde and paraffin embedded. At 75 dGA, OB ewes and their fetuses were ~50% and ~25% heavier

($P < 0.05$), respectively, than C ewes and fetuses. Fetal pancreatic tissue sections were incubated with guinea pig anti-porcine insulin or rabbit anti-MIF antibodies at 4° C overnight, then with fluorescent labeled 2° antibodies: Rhodamine labeled goat anti-guinea pig or AlexaFluor 488 labeled goat anti-rabbit for 60 min at 22° C. By evaluating immunostaining of both insulin positive cells and MIF positive cells, we determined that MIF is largely expressed within the cytoplasm of insulin positive beta cells. The protein level of MIF in pancreatic tissue was quantified via Western blot using a specific polyclonal antibody to MIF. The pancreatic MIF protein level was increased ($P < 0.05$) in fetuses of OB ewes compared with fetuses from C ewes. The increased pancreatic MIF expression in fetuses from OB ewes would be expected to potentiate insulin release and thus glucose uptake by fetal body tissues. NIH INBRE 1P20RR16474.

Key Words: Pancreatic Beta Cell MIF, Maternal Obesity, Sheep

475 Effects of soy-derived phytoestrogen and estradiol exposure on reproductive development in male neonatal pigs. K. Necaie*¹, K. Moulton¹, D. Christiansen¹, K. Walters¹, M. Crenshaw¹, C. Scanes², and P. Ryan¹, ¹Mississippi State University, Starkville, ²University of Wisconsin, Milwaukee.

While dietary phytoestrogens are known to mimic estrogenic effects on adult mammalian reproductive systems, there is little information on the effects of these compounds on fetal or neonatal reproductive development. The objective of this study was to evaluate the effects of soy-derived isoflavones on normal reproductive development in neonatal pigs. Yorkshire-Landrace crossbred sows (100 d gestation) were randomly assigned to a lactating diet (2.3 kg/sow/d) supplemented with either Novasoy 70, an isoflavone extract, (NOV, n=8) or without (CON, n=8). Mean BW for CON and NOV sows was 275.8 ± 7.0 kg and 278.8 ± 13 kg, respectively. Feed was top-dressed 2 x d with the NOV from d 100 of gestation to farrowing. Male neonatal pigs (NPs) were weighed on post natal day (PND) six then randomly assigned to one of four treatments (n=10 to 12/treatment): 1) control (vehicle), 2) estradiol (50 µg/kg BW/d) as a positive control, 3) low genistein dose (3 mg/kg BW/d) and 4) high genistein dose (9 mg/kg BW/d). NPs were dosed by oral gavage 2 x d for 7 d commencing on PND 7. Doses were adjusted daily according to BW change. On PND 14, NPs were euthanized and the testes, epididymi and seminal vesicles were recovered, grossly examined, and wet tissue weights recorded. Wet tissue weights were analyzed using GLM mixed model procedures. At PND 14 body weights did not differ ($P > 0.05$) between assigned NP treatments. Novasoy treatment of sows had no effect on testes, epididymal or seminal vesicles weights. However, estradiol treatment of NP increased seminal vesicle wet weight as a % of BW ($P = 0.075$), but there was no observed effects of estradiol or either genistein treatments on NP testes and epididymal weights. While no detrimental effects of estrogen exposure were observed in testes or epididymis, changes in seminal vesicle tissue weights demonstrates that oral exposure to estradiol but not genistein may alter reproductive development in male neonatal pigs. Thus, the neonatal pig may provide a useful model for assessing effects of dietary phytoestrogens on reproductive development in infants on soy-based formulas.

Key Words: Pigs, Phytoestrogens, Reproductive Development

476 Meta-analysis of progesterone supplementation during early pregnancy in cattle. G. E. Mann*, *University of Nottingham, Sutton Bonington Campus, Loughborough, UK.*

Progesterone is a critical hormone during early pregnancy in the cow. As a result a number of studies have investigated the effects of progesterone supplementation on pregnancy rate. An earlier meta-analysis of some of these studies revealed an overall improvement in pregnancy rate and marked effects of factors such as time of treatment (Mann and Lamming, 1999, *Reproduction in Domestic Animals* 34, 269-274). In this study an updated meta-analysis was carried out on 30 studies involving a total of 5262 control cows and 4726 progesterone treated cows. Treatment was by a variety of routes including intravaginal insert, subcutaneous implant and im injection. Data was analyzed by Chi square test with Yates correction. While the results of individual studies showed wide variations (range of 28.5% decrease to 34.4% increase), progesterone treatment did result in an overall increase ($P < 0.05$) in pregnancy rate of 2.4%. Treatment following natural or synchronized estrus resulted in an overall increase ($P < 0.001$) of 4.9% while treatment following synchronized ovulation did not result in any increase. In cows treated following unsynchronized ovulation, very early (d 0 to 2) or late (after d 10) initiation of treatment had no significant effect on pregnancy rate, while initiation of treatment between d 3 to 9 resulted in an overall 8.5% increase ($P < 0.001$) in pregnancy rate. This analysis demonstrates that progesterone supplementation is of no benefit following synchronized ovulation. However, if ovulation has not been synchronized, treatment results in improvements in pregnancy rate that are highly dependant on time of treatment.

Key Words: Cow, Progesterone, Pregnancy

477 Effect of endocannabinoid (EC) agonists on cow corpus luteum (CL) function in vitro. C. W. Weems^{*1}, Y. S. Weems¹, A. W. Lewis², D. A. Neuendorf², and R. D. Randel², ¹*University of Hawaii, Honolulu*, ²*Texas A&M University, Overton.*

Thirty to 40 % of pregnancies are lost during the first 3rd of pregnancy due to inadequate progesterone (P4) secretion. Loss of CL P4 secretion during the estrous cycle is via uterine secretion of PGF2 α . Cow CL secretion of PGE and PGF2 α , which are derived from arachidonic acid (AA) in phospholipids, increased linearly with time in culture with the PGE:PGF2 α ratio being 1:1. PGE1 or PGE2 are luteotropic and antiluteolytic in vitro and in vivo (C Weems et al. *The Vet J* 171:206, 2006; Y Weems et al. *Prostaglandins* 55:28, 55:359, 1988). EC are also derived from phospholipids and are associated with infertility, which could be via negative effects on implantation or CL function (Wang et al. *J Clin Invest* 116:2122, 2006; *Endo Rev* 27:427, 2006). The objective was to elucidate effects of EC1 or EC2 receptor agonists, antagonists or a fatty acid amide hydrolase (FAAH) inhibitor of EC catabolism on cow CL P4, PGE and PGF2 α secretion. Day-15 CL slices were weighed, randomized to treatments (100 ng/mL; n = 8) within cow, and incubated at 39.5 C in M199 1 hr without treatment and 4 and 8 hr with treatments. Treatments were: Vehicle (VEH), PGE1, PGF2 α , AM 251 (EC1 antagonist), AM 630 (EC2 antagonist), ACPA (EC2 agonist), IMMA (EC2 agonist), MAFP (inhibits FAAH catabolism of EC), MAFP+ACPA or MAFP+IMMA. Media collected at 4 and 8 h were analyzed for P4, PGE and PGF2 α by RIA. Data were analyzed by a 2X10 Factorial Design for ANOVA. P4 was increased by PGE1 ($P < 0.05$) and reduced ($P < 0.05$) by PGF2 α , ACPA, IMMA, MAFP, MAFP+ACPA, and MAFP+IMMA. PGE and

PGF2 α increased ($P < 0.05$) with time in VEH controls. PGE1 increased PGE and PGF2 α increased PGF2 α at 4 and 8 h ($P < 0.05$). ACPA, IMMA, MAFP, MAFP+ACPA and MAFP+IMMA lowered ($P < 0.05$) PGE secretion at 4 and 8 h and by AM251, AM 630 and PGF2 α at 8 h. AM 251, AM 630, MAFP+IMMA, ACPA, IMMA or MAFP+ACPA decreased ($P < 0.05$) PGF2 α at 4 and 8 h and by PGE1 at 8 h. Overall EC negatively affect cow CL function, The CL may be one site for EC decreased fertility.

Key Words: Cow, Corpus Luteum, Endocannabinoids

478 Peripheral concentrations of insulin are negativity correlated with cytochrome P450 3A activity and mRNA expression in dairy cows. C. O. Lemley*, L. R. Tager, K. M. Krause, and M. E. Wilson, *West Virginia University, Morgantown.*

Current dairy cow pregnancy rates to first service are very low, dramatically decreasing the potential profits to the industry. Dairy cow pregnancy rates have been improved with progesterone supplementation. Low peripheral concentrations of progesterone may be due to deficiencies in luteal secretion and/or excessive hepatic catabolism via cytochrome P450 enzymes. The objectives of the current experiment were to determine any association between plasma insulin concentrations and hepatic cytochrome P450 activity and/or mRNA expression. Six lactating Holstein dairy cows (123 \pm 38 DIM) were fed one of three diets (14 d experimental period) utilizing a Latin rectangle design. Diets were formulated to be isocaloric and isonitrogenous, while causing differential insulin secretion (2% propylene glycol diet, 15.1% corn starch diet or a high fiber diet). Energy balance and DMI on d 10-14 were similar among cows fed the three different diets. On d 12 a jugular blood sample was collected prior to the morning feeding at 0 hr, and then at 1, 2, 3, 4, 5, 6, 8, and 10 hr post-feeding for determination of insulin concentrations. On d 14 a liver biopsy was collected 3 hr post-feeding and used for determining cytochrome P450 2C and 3A activity and mRNA expression. Insulin response for each cow over the 10 hr blood sampling period was used to generate area under the curve (AUC) values. A correlation analysis revealed a significant negative correlation between cytochrome P450 3A activity and insulin AUC ($r = -0.49$, $P < 0.05$), as well as cytochrome P450 3A mRNA expression and insulin AUC ($r = -0.50$, $P < 0.05$). However, neither cytochrome P450 2C activity nor mRNA expression were correlated with insulin AUC. In conclusion we have demonstrated a significant negative correlation between cytochrome P450 3A activity and mRNA expression with insulin AUC, supporting the notion that feedstuffs that stimulate insulin secretion may be useful in decreasing excessive progesterone catabolism.

Key Words: Insulin, Cytochrome P450, Progesterone Catabolism

479 Biostimulatory effect of bulls alters characteristics of cortisol concentration patterns before resumption of ovulatory activity in postpartum, anovular, suckled beef cows. S. A. Tauck*, J. R. Olsen, J. R. C. Wilkinson, and J. G. Berardinelli, *Montana State University, Bozeman.*

The objective was to determine if bulls alter temporal patterns of cortisol concentrations in postpartum, suckled beef cows before resumption of ovulatory activity (ROA). The null hypotheses were that interval to

ROA and characteristics of cortisol patterns do not differ between cows exposed or not exposed to bulls. At 72 ± 3.5 d (\pm SE) postpartum, anovular cows were assigned randomly to be exposed (BE; n=8) or not exposed (NE; n=5) to bulls for 33 d in separate pen areas. Day 0 was designated as the start of bull exposure and BE cows had been exposed to bulls for 6 d before the initiation of the intensive sampling period. Blood samples were collected daily via an indwelling jugular catheter from each cow at 15-min intervals between 1000 to 1400 h over a 10-d interval (d 7 to 16) for assay of cortisol. Interval from d 0 to ROA, mean cortisol, and pulse frequency, amplitude, and duration, and inter-pulse interval were analyzed by ANOVA. Interval to ROA was shorter ($P < 0.05$) for BE (11.4 d) than NE (21 d) cows. Mean cortisol and characteristics of cortisol patterns did not differ ($P > 0.10$) between BE and NE cows from D 7 to 16. However, characteristics of cortisol patterns in BE cows differed ($P < 0.05$) before and after ROA. Five BE cows and only one NE cow began to cycle before or during the sampling period. Before the initiation of cycling activity cortisol pulse frequency was lower ($P < 0.05$) in BE cows than NE cows. Mean cortisol and cortisol pulse duration were correlated positively ($P < 0.05$) with interval to ROA in BE cows, while only mean cortisol was correlated positively ($P < 0.05$) with interval to ROA in NE cows. In conclusion, bull exposure decreased cortisol pulse frequency and lengthened cortisol pulse duration before ROA. The results indicate that changes in characteristics of cortisol concentrations precede the biostimulatory effect of bulls to induce resumption of ovulatory activity in postpartum, anovular, suckled beef cows.

Key Words: Bull Biostimulation, Cortisol, Postpartum

480 Effects of oro-nasal administration of androstadienone on characteristics of cortisol concentrations in postpartum, suckled beef cows. J. R. C. Wilkinson*, S. A. Tauck, J. R. Olsen, and J. G. Berardinelli, *Montana State University, Bozeman.*

The objective was to determine if oro-nasal administration of androstadienone changes characteristics of cortisol concentration patterns in postpartum, anovulatory beef cows. The hypotheses were that characteristics of cortisol concentration patterns do not change before, during, or after exposure to androstadienone in anovular cows. To test these hypotheses, three primiparous, crossbred cows were fitted with indwelling jugular catheters 2 d before the start of the experiment (92 ± 5.9 d after calving). Treatments were: oro-nasal administration of 0.9% saline on day 1 (S1); androstadienone (Andro; 10 mg/mL 0.9% saline) on day 2 (Andro); and 0.9% saline on day 3 (S2) for 4 h over 3 consecutive days. Oro-nasal exposure consisted of three vigorous sprays: one in each nostril and one on the top of the nose, given each h over a 4-h period each d. Samples were collected at 15-min intervals for 4 h from 1000 to 1400 h each day. Characteristics of cortisol patterns (mean, baseline, pulse frequency, amplitude, and duration) were analyzed by ANOVA. Mean, baseline, and pulse amplitude in cows did not differ ($P > 0.10$) among treatments. Pulse frequency was greater ($P < 0.05$) in cows during the S1 (1 pulse/h) and S2 (1 pulse/h) treatments than in cows during the Andro treatment (0.5 pulses/h). Pulse duration did not differ ($P > 0.10$) among cows treated with S1 and S2 or S2 and Andro. However, pulse duration was longer ($P < 0.05$) when cows were given Andro (75 min) than when given S1 (46.8 min). Androstadienone, administered oro-nasally to postpartum cows did not alter mean, baseline, and pulse amplitude of cortisol. However, androstadienone exposure appeared to decrease pulse frequency and increase the duration of pulses of cortisol. These preliminary results indicate that androstadienone may be a putative pheromone involved with the biostimulatory effect of bulls on resumption of ovulatory activity in postpartum, suckled beef cows.

Key Words: Bull Biostimulation, Postpartum, Pheromone