Physiology and Endocrinology III

TH332 Exogenous enzymes and *Salix babylonica* extract affects cellular immune response in growing lambs. N. Rivero[1], A. Z. M. Salem*[1], C. G. Penuelas[1], M. G. Ronquillo[1], H. Gado[2], and N. E. Odongo[3], *[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de Mexico, Mexico, [2]Animal Production Department, Faculty of Agriculture, Ain Shams University, Qalubia, Egypt, [3]Animal Production and Health Section, International Atomic Energy Agency, Vienna, Austria.*

The aim of this study was to determine effects of an exogenous enzyme of ZADO and *S. babylonica* L. extract on cellular immune response. Twenty 6- to 8-mo-old Suffolk lambs of 24 ± 0.3 kg BW were used. After 2 wk of adaptation to a basal diet, of 70% corn silage and 30% commercial concentrate (DM basis), the lambs were weighed and randomly distributed into 4 groups (n = 5 per group) using a completely randomized design and data of the immunological parameters was analyzed using the MIXED procedure of SAS with repeated measures. Enzyme product of ZADO is commercially available multi-enzyme feed additive in a powder form produced from *Ruminococcus flavefaciens* and manufactured by the Academy of Scientific Research and Technology in Cairo, Egypt. The feeding treatments for lambs were (1) Control: basal diet only; (2) EZ: Control diet plus 10 g of exogenous enzyme; (3) SB: Control diet plus 30 mL of *S. babylonica* L. extract; and (4) EZSB: Control plus 10 g exogenous enzyme and 30mL of *S. babylonica* L. extract. The daily dose of *S. babylonica* L. extract was given orally before the morning feeding while the exogenous enzyme was fed to the lambs mixed with 200 g of concentrate for 20 min and then mixed with the rest of the concentrate and offered for 1h before ad libitum feeding of corn silage. The experiment was 60 d in duration. Blood samples were collected on d 0, 15, 30, 45 and 60 from each animal via jugular venipuncture and flow cytometry analysis was used to assay numbers of T-helper lymphocytes (Th), cytotoxic-T lymphocytes (Tc), granulocytes and monocytes. In general, treatments had no effect on any of the parameters measured, but sampling time decreased Th (5.6 vs. 6.6, SEM = 0.35, linear and cubic effect, $P < 0.01$); and increased Tc (43.7 vs. 37.8, SEM = 1.04, cubic effects, $P < 0.01$), granulocytes (43.3 vs. 38.4, SEM = 0.57, linear and cubic effects, $P < 0.01$) and monocytes (6.2 vs. 5.9, SEM = 0.39, linear and cubic effects, $P < 0.01$). Results suggest that addition of exogenous enzyme and/or *S. babylonica* L. extract have immunosuppressive effects only during the first 15 d on as reflected by the treatment × day interaction.

Key Words: exogenous enzyme, immune response, lamb

TH333 Effects of intravenous β-hydroxybutyrate on the mRNA abundance of genes related to metabolism and immune response in hepatic and mammary tissue in dairy cows. M. Zarrin*[1,2], H.A. van Dorland[1], O. Wellnitz[1], and R.M. Bruckmaier[1], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Department of Animal Science, Yasouj University, Yasouj, Iran, [3]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland.*

Increased ketone body synthesis is a frequent metabolic adaptation during negative energy balance (NEB) at simultaneously high glucose consumption by the mammary gland after parturition in high yielding dairy cows. Beyond various metabolic changes the immune system is impaired during NEB and hyperketonemia. Our objective was to study the effects of a 48 h β-hydroxybutyrate (BHBA) infusion on the mRNA abundance of candidate genes related to metabolism and immune response in mammary and hepatic tissue in mid-lactation dairy cows. Thirteen cows were allocated to either intravenous Na-DL-β-OH-butyrate infusion (HyperB, n = 5) to achieve elevated plasma BHBA concentrations (1.7 mmol/L), or saline (0.9%) infusion (Control, n = 8). Liver and udder biopsies were taken one week before the start of the infusion and 48 h after the start of the infusion. Differences of mRNA abundance before and at the end of BHBA infusion were calculated and were tested for significance by a general linear model (GLM) including treatment (BHBA or NaCl) as fixed effect. The mRNA abundance of serum amyloid A (SAA) and haptoglobin (Hp) in the liver increased in both groups. BHBA infusion did not affect mRNA abundance of SAA, Hp, RANTES, and tumor necrosis factor α (TNFα) in the liver, and of acetyl-CoA carboxylase, citrate synthase, fatty acid synthase, β-hydroxybutyrate dehydrogenase 1 and 2, and succinyl-CoA: 3-ketoacid-coenzyme A transferase 1, mitochondrial (OXCT1) in mammary tissue. However, mRNA abundance of SAA in mammary tissue increased (8.97%) during BHBA infusion ($P < 0.01$). Hp mRNA abundance tended to increase (4.7%) during infusion in HyperB, and tended to decrease (1.77%) in the control group ($P = 0.07$). The results indicate that elevated plasma BHBA does not affect metabolism in the mammary gland and immune responses in the liver at a mRNA level. However, effects of BHBA on the mammary immune response could be related to increased susceptibility to infection during hyperketonemia.

Key Words: metabolism, hyperketonemia, immune response

TH334 Effects of bovine plasma handling and storage protocols on concentrations of haptoglobin and ceruloplasmin. P. G. M. A. Martins*, P. Moriel, and J. D. Arthington, *University of Florida, Institute of Food and Agricultural Sciences, Range Cattle Research and Education Center, Ona.*

Our objectives were to evaluate the effects of repeated freezing and thawing cycles, and different storage temperatures on concentrations of haptoglobin and ceruloplasmin using colorimetric procedures within biochemical assays. Briefly, haptoglobin concentrations were assessed via the measurement of haptoglobin/hemoglobin complexing by estimating differences in peroxidase activity, and ceruloplasmin concentrations, via estimation of oxidase activity. Blood samples were collected from 12 Brangus-crossbred steers on d 3 after vaccination against *Mannheimia haemolytica* (One Shot, Pfizer Inc. New York, NY). Blood samples were allocated to 1 of 5 handling protocols: (1) plasma samples were frozen, and thawed only on the day of analysis; (2) 24-h; blood samples were stored at 4°C for 24 h, and plasma was harvested, frozen, and thawed on the day of analysis; (3) 1-time; 1 wk before analysis, plasma samples were thawed for 1 h and re-frozen; (4) 2-time; 1 and 2 wk before analysis, plasma samples were thawed for 1 h and re-frozen; (5) 3-time; 1, 2, and 3 wk before analysis, plasma samples were thawed for 1 h and re-frozen. Each handling scenario was assessed at 1 and 7 mo of storage and at freezing temperatures of −20 and −80°C. Data were analyzed using the MIXED procedure of SAS. Concentrations of both proteins analyzed at 7 mo after blood sampling were greater ($P = 0.01$; 11% and 18% increase for haptoglobin and ceruloplasmin) than results from analysis conducted 30 d after blood sampling, irrespective of thawing and refreezing protocol. Samples subjected to 24-h storage at 4°C, before centrifugation and plasma harvest, had greater haptoglobin concentrations compared with 1, 2, and 3-time handling protocols ($P < 0.05$; 1.07 vs. 0.95, 0.94, and 0.95 mg/mL). For ceruloplasmin, a stor-

age temperature effect was detected with plasma concentrations stored at −80°C being greater than −20°C ($P = 0.05$; 30.1 vs. 28.2 mg/dL). In conclusion, plasma collection protocol, storage time, and storage temperature appear to affect the results of biochemical assays aimed at the quantification of bovine haptoglobin and ceruloplasmin.

Key Words: acute phase protein, beef cattle, stability

TH335 Purinergic signaling gene network expression in bovine polymorphonuclear neutrophils during the peripartal period. J. Seo, J. S. Osorio*, and J. J. Loor, *University of Illinois, Urbana.*

An effective immune response relies on efficient activation of polymorphonuclear neutrophils (PMN). PMN release cellular adenosine triphosphate (ATP) in response to exogenous stimuli such as inflammatory mediators. The peripartal period is characterized by marked changes in inflammatory status that are functionally related with impaired immune responses in the cow. We evaluated the mRNA expression of genes associated with the purinergic signaling in PMN during the peripartal period. Seven multiparous Holstein cows were dried off at d −50 relative to expected calving and fed a controlled-energy diet (NEL = 1.24 Mcal/kg of DM) at intakes to meet and not greatly exceed 100% of NRC requirements. All cows were fed a common lactation diet after calving. RNA from PMN collected at −10, 3, and 21 d relative to parturition were used to measure expression of 22 genes associated with adhesion to endothelium, chemoattractant binding at the plasma membrane, and purinergic signaling. The ANOVA model had day as the fixed effect and cow as the random effect. Differences between days were significant at a $P < 0.05$. The expression of P2RY2, a G-protein coupled receptor of adenosine, increased 2-fold at parturition compared with −10 d suggesting that ATP plays a role in the amplification of chemotactic signals. The expression of genes encoding cell adhesion (SELL and SELPLG), chemoattractant receptors (C5AR1, CXCR1, CXCR, and PTAFR), and adenosine receptors (ADORA1 and ADORA3) decreased at parturition compared with −10 d. The expression of ADORA2A, which is associated with immunosuppression of PMN, was 1.5-fold greater at 3 d than −10 d. The increase in expression of adenosine uptake channels (SLC29A1 and SLC29A2) and ADA after calving suggested that the concentration of extracellular adenosine might be sharply increased compared with pre-partum. This might be a cause of immunosuppression. Overall, our results suggested that the reduction in key immune responses such as cell adhesion and chemotaxis by bovine PMN are partly a function of changes in mRNA expression of genes associated with purinergic signaling.

Key Words: inflammation, PMN, transition cow

TH336 Dynamics of TLR-4 signaling in bovine neutrophils during the periparturient period. M. G. H. Stevens*[1,2], X. Boulougouris[1], C. Rogiers[1], T. McFadden[2], L. Peelman[1], B. De Spiegeleer[1], L. Duchateau[1], and C. Burvenich[1], *[1]Ghent University, Ghent, Oost-Vlaanderen, Belgium, [2]University of Missouri, Columbia.*

During the periparturient period mature blood neutrophils have a lower performance in migration, phagocytosis and respiratory burst assays. These functional alterations have been correlated to an increased risk for severe *E. coli* mastitis. We hypothesize that alterations in TLR-4 signaling may be involved in the functional dysregulation of neutrophils. In this preliminary study blood neutrophils of 11 cows were isolated at −14, 3 and 14 d relative to parturition and stimulated with LPS (10µg/mL). Total RNA was extracted and the expression of TLR-4 related genes was quantified by RT-qPCR. Data were analyzed based on a mixed model with stimulation and lactation stage and their interactions as categorical fixed effects and cow as random effect. Time relative to parturition had an effect ($P < 0.05$) on the expression of TLR4, CD14, RELA, MAPK1, MAPK3, IL8 and IL1β but not on TNFα. The expression of TLR4, CD14 and RELA was on average 25% lower at d-14 compared with d3 and d14. Compared with d-14, IL8 expression decreased with 60% at d3 and increased with 650% at d14. The expression of MAPK1 and MAPK3 increased with 22% at d3 but returned to prepartum values at d14. IL1β expression was similar at d-14 and d3 but increased with 22% at d14. LPS stimulation increased the expression of TLR4, CD14, RELA, IL1β and TNFα ($P < 0.05$) and decreased the expression of MAPK1 and MAPK3 ($P < 0.05$) but had no effect on IL8. The expression of TLR4, IL8, IL1β and TNFα upon LPS stimulation didn't change as a function of time. The effect of LPS stimulation on the expression of CD14, MAPK1 and MAPK3 was lowest at d-14 but was similar between d3 and d14. The effect of LPS on RELA expression was similar between d-14 and d3. At d14 neutrophil expression of RELA increased with 75% compared with d-14. This preliminary study suggests that the constitutive expression of genes related to the TLR-4 pathway changes as a function of time relative to parturition. Moreover, responsiveness of neutrophils to LPS may be altered during the periparturient period due to changes in the activation of the TLR-4 signal transduction pathway.

Key Words: neutrophil, periparturient period, TLR-4

TH337 Expression of niacin receptor GPR109A in bovine oocytes and preimplantation embryos and effect of addition of niacin during embryo culture on development following exposure to heat shock. J. Block[1,2], A. Ruiz[2], A. M. Reeg[1], L. K. Mamedova[3], B. J. Bradford[3], and T. R. Bilby*[4], *[1]OvaTech LLC, Gainesville, FL, [2]Department of Animal Sciences, University of Florida, Gainesville, [3]Department of Animal Sciences and Industry, Kansas State University, Manhattan, [4]Texas A&M AgriLife Research and Extension, Texas A&M System, Stephenville.*

Objectives were to determine whether niacin receptor GPR109A is expressed in bovine oocytes and preimplantation embryos, and to determine whether addition of niacin during embryo culture improves embryo development in the presence or absence of heat shock. In exp. 1, immature cumulus-oocyte complexes were collected from abattoir derived ovaries and used to produce embryos in vitro. Pools of immature oocytes denuded of cumulus cells and embryos at the 2-cell, 8-cell, ≥ 16-cell and blastocyst stages were harvested at the time of collection and 28–32, 68–74, 118–124 and 166–172 h post-insemination, respectively over 2–4 replicates. Total RNA was isolated and quantitative real-time RT-PCR was performed using primers designed for GPR109A and β-actin. Relative mRNA abundance was quantified by the delta C_t method with β-actin used to normalize values. There was a significant ($P < 0.05$) effect of stage of development on the relative expression of GPR109A, with embryos at the ≥16-cell stage having higher levels of GPR109A than immature oocytes or embryos at other stages. In exp. 2, embryos were produced in vitro using abattoir-derived oocytes. At d 3 after insemination, ≥ 8-cell embryos were harvested and randomly cultured with or without 100 µ*M* niacin for 9 h, then exposed to either control (38.5°C) or heat shock (41.0°C) treatments for 24 h. All embryos were then returned to 38.5°C and cultured until d 8. There was a tendency ($P < 0.08$) for niacin to reduce the proportion of ≥8-cell embryos that became blastocysts on d 8 (59.9 vs. 45.2 ± 4.1%). Heat shock significantly ($P < 0.05$) reduced the proportion of ≥8-cell embryos that became blastocysts on d 8 (61.7 vs. 43.4 ± 5.5%). There was no interaction between niacin and heat shock affecting embryo development. Results

indicate that niacin receptor GPR109A is expressed in bovine embryos produced in vitro. However, addition of niacin to embryo culture did not improve embryo development to the blastocyst stage regardless of whether embryos were exposed to heat shock.

Key Words: niacin, receptor, embryo

TH338 Prepartum body condition score changes and the secretion of acute phase proteins in dairy cows. P. Montagner[1,2], E. Schwegler[1,2], M. M. Weschenfelder[1,2], A. R. Krause[1,3], J. Alvarado[1,2], A. S. Maffi[1,2], C. C. Brauner[1,3], A. Schneider[1,4], E. Schmitt*[1,2], E. G. Xavier[5,2], C. F. Martins[1,2], V. R. Rabassa[1,2], F. A. B. Del Pino[1,3], and M. N. Correa[1,2], *[1]Center for Research, Teaching and Extension in Animal Science (NUPEEC), Pelotas, RS, Brazil, [2]Department of Clinical Veterinary, Federal University of Pelotas (UFPEL)-BRA, Pelotas, RS, Brazil, [3]Department of Animal Science, (UFPEL - BRA), Pelotas, RS, Brazil, [4]Departament of Nutrition (UFPEL - BRA), Pelotas, RS, Brazil, [5]Granjas 4 Irmaos, Rio Grande, RS, Brazil, [6]Center for Agroforestry Research of Rondonia - Embrapa CPAF, Rondonia, RO, Brazil.*

The aim of this study was to investigate the effect of body condition (BCS) score changes during the prepartum period on the concentration of acute phase proteins and metabolic parameters in dairy cows. Evaluation of BCS was performed on 20 pregnant Holstein dairy cows (on a 5-point scale with quarter-point divisions), from a commercial herd kept in a semi-extensive system in southern of Brazil. The cows were divided into 2 groups: cows that increased BCS (+0.25) from the third to the first week before the expected calving date (UP-BCS; n = 11) or cows that decreased BCS (−0.8) (LO-BCS; n = 9) in the same time frame. Blood samples were collected from the coccygeal vein on the d 23, 14, 7 and 3 prepartum, on the calving day and at d 3, 6, 9, 16 and 23 postpartum to evaluate the concentrations of nonesterified fatty acids (NEFA), glucose (GLU), albumin (ALB), haptoglobin (Hp) and paraoxonase (PON). Milk yield was recorded daily, and a 5-d average was generated, from 16 to 41 d in milk. Statistical analysis was performed using the SAS software, using the MIXED procedure for repeated measures. Average milk yield was higher for UP-BCS cows ($P < 0.01$; 27.4 kg/d vs. 24.4 kg/d). The UP-BCS cows had higher serum concentration of PON and ALB in the pre and postpartum periods ($P < 0.05$), while the LO-BCS group had higher levels of Hp in both periods ($P < 0.05$). Other variables (GLU and NEFA) were not different between groups ($P > 0.05$). These results indicate that BCS loss in the pre-partum period can affect the pattern of secretion of acute phase proteins in dairy cows in the transition period. In sum, our results indicate that higher BCS loss is associated to increased secretion of HP and reduced secretion of PON and ALB, which can be associated to increased risk of disease development.

Key Words: body condition score, haptoglobin, paraoxonase

TH339 Relationships of birth weight traits with age at first estrus and number of ovulations in Landrace-Duroc-Yorkshire gilts. C. A. Lents*, L. A. Rempel, T. Wise, and D. Nonneman, *U.S. Meath Animal Research Center, Agricultural Research Service, United States Department of Agriculture, Clay Center, NE.*

Selection for increased litter size has resulted in greater within-litter variation in piglet birth weight and a reduction in litter average birth weight; believed to be associated with intrauterine growth restriction as a result of limitations in uterine capacity. This leads to increased preweaning mortality, reduced growth performance, decreased muscle fiber number and reduced carcass quality. Low birth weight gilts have more primordial and fewer primary and secondary follicles suggesting that variation in average litter birth weight could negatively affect reproductive traits. The objective of this study was to examine the effects of birth weight traits with age at puberty and number of ovulations in gilts. Age at puberty, the first standing estrus in the presence of a mature boar, was determined for 2,187 gilts beginning at approximately 140 d of age. The number of ovulations for 2,173 gilts was determined during postmortem examination by counting the number of corpora lutea on the ovary after the first or second parity. Partial correlation coefficients for total born, total born alive, litter average birth weight, CV of litter average birth weight, individual birth weight, and deviation of individual birth weight from litter average birth weight with age at first estrus and number of ovulations were estimated using a model that fit season and line as fixed effects and sire as a random effect. Average age at first estrus and number of ovulations was 195.1 ± 0.4 d of age and 16.3 ± 0.1 ovulations, respectively. Litter average birth weight ranged from 0.79 to 2.45 kg with CV ranging from 23.5 to 44.7%. There were no significant correlations for age at first estrus with any of the birth weight traits examined. The number of ovulations was weakly correlated ($r = 0.08$, $P < 0.001$) with individual birth weight but not any of the other birth weight traits. These data do not support the concept that differences in average litter birth weight contributes to variation in pubertal age or ovulation rate in pigs.

Key Words: birth weight, ovulation rate, puberty

TH340 Determining the effect of scrotal insulation on sperm production in the boar. K. M. Gibbs*, J. R. Schindler, and J. J. Parrish, *University of Wisconsin-Madison, Madison.*

The objective of this study was to develop a model of heat stress in the boar using scrotal insulation to determine which stages of development were most susceptible to damage and apoptotic loss. The experiment utilized sacks that were adhered to the scrotum to produce a localized heat insult. Sacks were either insulated with batting and foil vapor barrier or were of the same design but without insulation material as a sham treatment. Semen was collected and analyzed for motility and total sperm output from the boars on a Monday, Wednesday, and Friday schedule leading up to the treatment and 6 weeks post-treatment. Scrotal sacks, non-insulated or insulated, were adhered to the testes and temperature loggers were attached to the scrotum to measure scrotal temperature over the 48 h treatment period. A significant difference in average temperature was achieved during the treatment between the insulated group (n = 5) and non-insulated group (n = 5) (mean ± SEM, 33.9 ± 0.33°C vs. 32.1 ± 0.43°C; $P < 0.05$). Semen samples were evaluated for motility and total sperm output. Motility was determined using computer assisted semen analysis with Hamilton Thorne motion analysis system. The non-insulated group (n = 4) showed no significant difference in motility compared with the control days (mean ± SEM, 96 ± 0.52%; $P > 0.05$). However, the insulated group (n = 4) showed a significant decrease in motility for d 28,30,33, and 35 compared with the control days (mean ± SEM, 78 ± 5.00%, 69 ± 6.76%, 66 ± 11.85%, 69 ± 9.86% vs. 95 ± 0.89%; $P < 0.05$). Total sperm output was not different for the non-insulated group (n = 5) compared with the control days (mean ± SEM, 35 ± 3 billion; $P > 0.05$). However, there was a trend indicating a decrease in sperm cell output on d 33 for insulated boars compared with the control days (mean ± SEM, 24 ± 6 billion vs. 35 ± 3 billion; $P = 0.06$). The data suggests that an average temperature increase of 1.8°C of the testes can have damaging effects to boar semen quality. Based on the known spermatogenic timeline in the boar, the main cell stage affected by the scrotal insulation treatment was the primary spermatocyte.

Key Words: semen, heat stress, swine

TH341 Influence of fat supplementation on leptin and LH concentration in Nelore heifers. R. S. Cipriano*, M. C. V. Miguel, H. F. Costa, J. S. Souza, L. M. Pavanello, J. L. C. Delfino, D. Giraldo-Arana, D. M. Pinheiro, and G. P. Nogueira, *UNESP, Animal Endocrinology Laboratory, DAPSA, FMVA, Aracatuba, Sao Paulo, Brazil.*

The aim of this study was to verify whether rumen protected fat supplementation, after weaning increase leptin and LH concentration in Nelore heifers (*Bos taurus indicus*). Contemporary heifers (n = 30) with 167 ± 13 kg and 9 mo were sorted into 3 experimental groups: Control Group (CG, n = 10), sugar cane bagasse plus 4.2 kg concentrate and 500g of ground corn; Fat Group (FG, n = 10), sugar cane bagasse, plus 4.2 kg of concentrate plus 200g of Megalac-E per animal (rumen protected fat); and Excess Group (EG, n = 10), sugar cane bagasse plus 4.2 kg of concentrate, 500 g of ground corn and 200g of Megalac-E per animal per day. After an adaptation period, animals remained under nutritional treatments for 92 d (13th to 16th month of age). Blood samples were collected every 4 d, between 9th and 18th month of age, and every 7 d from the 18th until 20th month of age for leptin and LH quantification. The results were evaluated by repeated measures ANOVA and the Duncan's test was the post-test of SAS. After treatment, the EG presented the lowest LH concentration total area (31.18 ± 14.91 ng/mL × day), P = 0.06) in comparison with CG (63.20 ± 33.89 ng/mL × day) and FG (82.90 ± 48.50 ng/mL × day), from samples collected every 4 d. There was no difference (P > 0.05) in the total area and in the LH concentration maximum amplitude between the groups before and during the nutritional treatments. Before treatment, the EG showed higher leptin concentration amplitude (P = 0.08, 2.99 ± 1.62 ng/mL) than CG (1.71 ± 0.77 ng/mL). There was no difference in leptin concentration amplitude between groups either before, during or after treatment (P = 0.49, 2.46 ± 0.96, 2.03 ± 1.26 and 1.86 ± 1.20 ng/mL). The FG and EG showed smallest leptin amplitude during and after treatment combined compared with before (FG: 2.03 ± 1.26 vs. 2.87 ± 1.57 ng/mL, P = 0,052; EG: 1.86 ± 1.20 vs. 2.99 ± 1.62 ng/mL, P = 0.03), and the CG showed greatest leptin amplitude after and during combined when compared the period before treatment (2.46 ± 0.96 vs. 1.71 ± 0.77 ng/mL, P = 0.03). We concluded that excess treatment decreased the LH concentration area and fat treatment decreased leptin amplitude after supplement period.

Key Words: fat, leptin, LH

TH342 Effect of supplementation of distillers grains during early pregnancy on reproductive performance of beef cows. A. M. Schreiner[1], P. M. Fricke*[2], E. J. Cretney[2], A. E. Radunz[1], and J. S. Luther[1], *[1]University of Wisconsin-River Falls, River Falls, [2]University of Wisconsin-Madison, Madison.*

Crossbred Angus cows (initial BW = 671 ± 8.8 kg; age = 6.3 ± 0.29 yr) were used to evaluate the effects of corn distillers' grains plus solubles (DG) supplementation during early pregnancy on reproductive performance. Cows were randomly assigned to 1 of 3 treatments (n = 33 cows/trt): no supplementation (CON); low supplementation of 2.7 kg of dried distillers grains/hd/d (LDG); and high supplementation of 5.4 kg of DDGS/hd/d (HDG). During the supplementation period, cows were housed in separate pastures of similar forage quality and DG supplementation contained 50% dried DG and 50% modified wet DG on a DM basis. Supplementation of DG began 7 d before artificial insemination (AI, d 0) and ended 20 d later. Body weight (BW) and body condition scores (BCS) were collected at the start (d −7) and end of supplementation (d 20). Blood samples were collected for progesterone (P4) analysis on d 4, 6, 8, 11, 13, 15, 18, and 20. B-mode ultrasonography was used to determine diameter of the dominant follicle (d −1) and corpus luteum (CL; d 13), and pregnancy status (d 33). Cows fed LDG and HDG had greater (P < 0.001) BW and greater (P < 0.05) BCS at the end of supplementation compared with CON cows. No difference (P > 0.05) was observed in follicle diameter among treatments. Cows fed HDG had a greater (P < 0.05) CL diameter compared with cows fed CON or LDG. From 4 to 20 d after AI, area under the curve for plasma P4 was greater in cows fed LDG (P < 0.02) and HDG (P < 0.05) compared with CON cows. Pregnancy rates among CON (73%), LDG (70%) and HDG (79%) cows were similar (P = 0.69). Supplementation of DG during early pregnancy improved BW and BCS and increased plasma P4 concentrations, but pregnancy rates did not differ among treatments.

Key Words: distillers grain, beef cattle, reproduction

TH343 Effect of capsicum oleoresin on proliferation and cytokine production in bovine peripheral blood mononuclear cells. J. Oh*[1], S. Walusimbi[1], A. N. Hristov[1], J. Pate[1], and D. Bravo[2], *[1]Department of Animal Science, The Pennsylvania State University, University Park, [2]Pancosma, Geneva, Switzerland.*

The objective of this experiment was to investigate the effect of capsicum oleoresin (CO) on proliferation of, and cytokine production in, activated and non-activated bovine peripheral blood mononuclear cells (PBMC). Peripheral blood mononuclear cells were obtained from blood collected from dry Holstein cows (n = 4). The cells were cultured in the presence or absence of immune cell activating compounds, phorbol 12-myristate 12-acetate (PMA, 50 ng/mL) and ionomycin (1 μg/mL), and treated with CO at 4 concentrations, 0 (control), 40, 160, and 320 μg/mL. Cell viability and proliferation were determined by flow cytometry using propidium iodide (PI) and carboxyfluorescein diacetate succinimidyl ester (CFSE), respectively. There was no effect of CO on cell viability in activated or non-activated PBMC. However, CO at 320 μg/mL increased (P < 0.001) the percent proliferating cells (64.9%) compared with the control (4.6%) in activated cells. Cytokine production was detected by intracellular staining and flow cytometry. Brefeldin A (BFA) was added to prevent the cells from releasing the cytokines into the media. Activated and non-activated PBMC treated with CO were labeled with antibodies against tumor necrosis factor α (TNF), interferon gamma (IFNG), and interleukin 10 (IL10). Compared with the control, CO at 160 and 320 μg/mL significantly increased (P = 0.04 and 0.01) the production of IFNG in non-activated (1.04 vs. 2.18 and 2.64% positive cells, respectively), but not in activated PBMC. There was no effect of CO (P = 0.19 to 0.65) on the production of TNF or IL10. In conclusion, CO at high concentrations induced both proliferation and IFNG production. It is suggested that CO may facilitate activation of the immune system in dairy cows.

Key Words: capsicum oleoresin, cytokine, cell proliferation

TH344 Growth and cardiovascular characteristics between birth and one month of age in dairy calves. B. E. Voelz*, H. M. Kerr, D. K. Hardin, K. A. Barton, C. O. Lemley, and J. E. Larson, *Mississippi State University, Mississippi State.*

Growth characteristics and cardiac function in a young calf may affect health and production characteristics later in life. The objective of this experiment was to determine if growth characteristics and cardiac measurements were correlated between 2 ages in a calf's life or between each other at one time point. Holstein (n = 30) and Jersey (n = 8) calves were evaluated at 2 d (±1 d) after birth (n = 38) and again at 1 mo (±4 d) of age (n = 27). Measurements at birth and at 1 mo of age included blood pressure, heart rate, heart girth, hip and wither height as well as carotid artery hemodynamics measured via Doppler ultrasonography [pulsatility index (PI), resistance index (RI), and vessel diameter]. From

these measurements, mean arterial pressure (MAP), pulse pressure (PP), mean velocity (MnV) and blood flow (BF) were calculated. The CORR procedures of SAS were used to analyze data; means (±SD) are presented. Heart girth (r = 0.774) and hip (r = 0.795) and wither height (r = 0.765) at birth were all ($P < 0.0001$) positively correlated with size measurements at 1 mo of age. Heart rates of calves at birth (128 ± 19 bpm) were significantly ($P < 0.01$) and positively correlated with MAP at 1 mo of age (r = 0.564; 93 ± 15 mmHg). Blood MnV and BF (373 ± 201 mL/min) at birth were ($P < 0.05$) negatively correlated with PP (r = −0.456; 48.6 ± 9.9 mmHg) and RI (r = −0.443; 0.855 ± 0.12), respectively, at 1 mo. RI (r = 0.461; $P < 0.05$) and PI (r = 0.372; $P = 0.06$) at birth were positively correlated with BF at 1 mo of age (426 ± 204 mL/min). Birth hip (r = 0.340) and wither height (r = 0.329) tended to be significantly ($P < 0.10$) and positively correlated with MAP at birth (79.8 ± 8.6 mmHg), indicating larger calves had greater arterial pressure. PI at birth ($P < 0.05$) and at 1 mo (2.8 ± 1.7; $P < 0.10$) were negatively correlated with BF at birth (r = −0.355) and 1 mo (r = −0.326), respectively, giving indication that these data correspond to previous research using Doppler ultrasonography. Further characterization of calf cardiovascular hemodynamics may allow researchers to examine potential neonatal outcomes associated with later life production characteristics.

Key Words: cattle, dairy, Doppler

TH345 Effect of decreased progesterone concentrations during follicular development on oocyte yield and quality. F. M. Abreu*[1], S. Kruse[2], L. H. Cruppe[1], R. S. Cipriano[1], M. L. Day[1], T. W. Geary[3], M. A. Coutinho da Silva[1], B. A. Hicks[4], D. S. Clark[4], and G. A. Bridges[2], *[1]The Ohio State University, Columbus, [2]University of Minnesota, Grand Rapids, [3]USDA ARS Fort Keogh, Miles City, MT, [4]Simplot Livestock Inc., Emmett, ID.*

The objective was to determine if decreased concentrations of progesterone (P4) during early follicular development would affect number and quality of oocytes recovered by transvaginal ultrasound-guided ovum pick-up (OPU). Ovulation was synchronized with the 5 d CO-Synch + CIDR program in postpubertal heifers in 2 groups (n = 18 per group) with d of the 2nd GnRH treatment designated as d 0. On d 5.5 all visible follicles in the ovaries were ablated. Heifers were stratified, within group, by estrous expression (yes or no), weight, age, and antral follicle count to receive either a new CIDR (high P4; H) or a previously used CIDR and 2–25 mg doses of PGF given 8 h apart (low P4; L) on d 5.5. On d 10.5 (OPU-1), all visible follicles were aspirated, new and used CIDR were replaced, and OPU was performed again on d 15.5 (OPU-2). Follicle stimulating hormone (FSH; 50 mg per dose) was administered on d 7.5, 8, 8.5 and 9 and d 12.5, 13, 13.5 and 14. Blood samples for P4 were collected at ablation, OPU-1, and OPU-2. Number of follicles aspirated was recorded at each OPU and oocytes were graded on a 1 to 6 scale (1 = ≥5 layers of compact cumulus and homogenous cytoplasm, 6 = denuded). Concentrations of P4, total follicles aspirated, total oocytes recovered, and oocyte quality were compared with the MIXED procedure of SAS. Concentrations of P4 did not differ on d 5.5, but were lower ($P < 0.01$) at OPU-1 and OPU-2 in the L (3.03 ± 1.92 and 2.00 ± 1.01 ng/mL, respectively) than in the H (5.47 ± 2.06 and 5.36 ± 1.60 ng/mL, respectively) treatment. Across OPU-1 and OPU-2, the L treatment had more ($P < 0.05$) total follicles aspirated (15.3 ± 1.1) and oocytes recovered (9.9 ± 1.2) than heifers in the H treatment (12.1 ± 1.0 and 6.4 ± 0.8, respectively). Furthermore, decreased P4 resulted in increased ($P < 0.05$) number of grade 1–3 oocytes collected per heifer (L: 7.78 ± 1.03, H: 4.81 ± 0.72). In conclusion, lesser P4 concentrations during follicular emergence and early development resulted in collection of a greater number of good quality oocytes per heifer by OPU when compared with heifers with greater peripheral P4 concentrations.

Key Words: progesterone, heifer, oocyte

TH346 Integrating nutritional and reproductive models to improve reproductive efficiency in dairy cattle. S. L. Shields* and J. P. McNamara, *Department of Animal Sciences, Washington State University, Pullman.*

Successful reproduction requires coordination among neural, endocrine and nutritional systems leading to ovulation, insemination and a uterine environment that allows embryonic growth and attachment. However, we still lack, in research and practice, a systems approach to integrating genetics and nutrition to improve reproduction. Therefore, our objective was to integrate 2 existing mechanistic, dynamic models of genetic, nutritional and reproductive processes in the dairy cow. A model of metabolism (Molly, UC Davis); which describes metabolism of glucose, VFA, and amino acids for fat and protein synthesis and degradation and milk component production, as well as energy transactions (ADP/ATP), was integrated with a model of reproductive processes which describes growth and decay of the follicles and corpus luteum, gonadotropin releasing hormone, follicle stimulating hormone, luteinizing hormone, progesterone, estrogen, oxytocin, and prostaglandin F2α over time. The 2 models were integrated at specific points based on available literature data: glucose and IGF-I affect rates of follicle stimulating hormone, luteinizing hormone, and follicular growth; higher glucose supply increases IGF-1 and increases follicular growth. Increasing circulating concentration of IGF1 by 20 ng/ml will increase follicular growth by 1 mm in 18 d (versus an average of 12 mm pre-ovulation), leading to an earlier ovulation. Increasing AtAdv (total ATP conversion to ADP) from 372 to 1488 moles/d decreases peak estrogen from 0.342 to 0.281. Increased metabolic rate from either increased milk production or feed intake decreases estrogen and progesterone concentration. The model responses to energy intake and milk production caused a pattern and direction of response in reproductive processes consistent with available data. This research model should be useful to frame specific hypotheses on control of reproductive processes by genetic and nutritional driven mechanisms and to help develop on-farm decision support tools.

Key Words: systems biology, reproduction, nutrition

TH347 Effect of maternal dietary fish oil supplementation on growth and physiological indicators of stress in pre- and post-weaned pigs. S. A. Lockwood*, H. G. Kattesh, C. J. Kojima, M. P. Roberts, G. M. Pighetti, and A. M. Saxton, *University of Tennessee, Knoxville.*

The aim of this study was to assess whether feeding sows a diet with 0.5% protected fish oil (PFO) 2 wk before farrowing through lactation (CON, n = 2; PFO, n = 4) can reduce the stress response of their offspring due to weaning. Colostrum and milk was obtained on d 0 and 20 of lactation. Upon weaning (d 27), 24 pigs from each treatment were blocked by BW and allocated in groups of 6 to nursery pens and fed a diet consistent with dam dietary treatment. Pigs were weighed on d 17, 27, 34, 41, and blood sampled on d 20, 27, 28, 34, and 41. Blood was analyzed for plasma cortisol (CORT) and corticosteroid-binding globulin (CBG) concentrations, and white blood cell (WBC), red blood cell (RBC), and differential WBC numbers. Free cortisol index (FCI) was calculated from plasma CORT and CBG (nmol CORT/mg CBG). Data were pre-adjusted for age difference by quadratic regression, then mixed model repeated measures ANOVA (SAS) was performed. Triglyceride

concentration was higher ($P = 0.03$) in milk compared with colostrum and was not different due to treatment. Pre- and post-weaning BW and RBC were not different ($P > 0.10$) among treatments. No treatment differences were detected for blood parameters measured on d 20. On d 27, CBG but not CORT was greater ($P < 0.01$) in PFO pigs resulting in a lower ($P < 0.01$) FCI (2.9 vs. 5.0 ± 0.4 nmol/mg). Upon weaning, all pigs exhibited an increase ($P < 0.01$) in CORT, which tended ($P = 0.08$) to be greater for the CON pigs. As a result of lower CBG concentration, the FCI for the PFO and CON pigs did not differ. By d 41, regardless of treatment, CORT returned to pre-weaning level and CBG reached its greatest concentration resulting in the lowest overall FCI value recorded ($P < 0.01$). The WBC was greater ($P < 0.01$) for PFO pigs on d 27 and 28 but less ($P < 0.01$) than that measured for CON by d 41. The N:L ratio measured on d 28 tended ($P = 0.06$) to be greater for the CON compared with the PFO pigs. Supplementation of the sows' diet with a PFO, beginning 2 wk before farrowing and throughout lactation, had minimal influence on modifying the pigs' stress response due to weaning.

Key Words: fish oil, pig, weaning

TH348 Effects of intrauterine infusion of *Trueperella pyogenes* on endometrial mRNA expression of genes associated with luteolysis in dairy cows. F. S. Lima*, J. E. P. Santos, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, N. Martinez, C. A. Risco, W. W. Thatcher, and K. N. Galvão, *University of Florida, Gainesville.*

Objective was to determine the effects of intrauterine (IU) infusion of *Trueperella pyogenes* (*T. pyogenes*) on endometrial mRNA expression of genes affecting the luteolytic cascade. Fifteen early postpartum healthy Holstein cows had the estrous cycle synchronized with on GnRH d0 followed by $PGF_{2\alpha}$ on d7 and GnRH on d9. Four days after ovulation was confirmed cows were allocated randomly to receive one of 3 treatments: TP (n = 5), IU infusion of 10 mL of saline solution containing 10^9 cfu/ml of *T. pyogenes* (formerly *Arcanobacterium pyogenes*); TNF (n = 5), IU infusion of 10 mL of saline solution containing 1 μg of tumor necrosis factor α (TNFα); and Control (n = 5), IU infusion of 10 mL of saline solution. Uterine biopsies were collected at 6, 12 and 24 h after treatment to evaluate the endometrial mRNA expression of TNF-α, Interleukin (IL) 1β, IL6, IL8, prostaglandin E synthase (PGES), PGFS and oxytocin receptor (OXR). RT-PCR was used to measure mRNA expression. MRPS15 was used as housekeeping gene. The MIXED procedure of SAS was used for statistical analysis. Gene expression of IL1β and IL6, PGES and PGFS was not affected by treatment, time or treatment by time interaction. However, TP cows had higher ($P < 0.05$) mRNA expression of IL1β and IL8 than TNF cows at 24 h. TNFα mRNA expression was lower ($P < 0.05$) for TP cows than TNF cows at 6 h. Overall OXR mRNA expression was higher ($P = 0.03$) for TP cows than Control cows. In conclusion, IU infusion of *T. pyogenes* did not consistently increase endometrial mRNA expression of genes involved in the luteolytic cascade; however, the endometrial expression of OXR was increased for cows infused with *T. pyogenes*.

Key Words: dairy cow, *Trueperella pyogenes*, gene expression

TH349 Correlations between PAG concentrations, pregnancy loss, and milk production in high producing Holstein cows. P. Mercadante*[1], C. Risco[2], and A. Ealy[1,3], *[1]University of Florida, Department of Animal Sciences, Gainesville, [2]University of Florida, Department of Large Animal Clinical Sciences, Gainesville, [3]Virginia Polytechnic Institute and State University of Florida, Department of Animal and Poultry Sciences, Blacksburg.*

Pregnancy-associated glycoproteins (PAGs) are produced by the ruminant placenta and secreted into the maternal circulation during pregnancy. Their concentrations in early gestation may indicate pregnancy failure in cattle. To determine if plasma PAG and P4 concentrations are associated with pregnancy loss and several production variables in high producing cows, 125 multiparous Holsteins were timed AI using semen from multiple sires. Transrectal ultrasonography was used to diagnose pregnancy on d 32, 46 and 74 of gestation. Blood was harvested to determine plasma concentrations of PAG and P4 at d 32. Data was segregated into groups based on whether cows maintained their pregnancies to term (n = 101) or lost their pregnancies between d 32 and 46 (n = 9), d 46–74 (n = 9) or after d 74 (n = 6). The CORR procedure of SAS was used to assess traits associated with PAG and P4 concentrations, GLM procedure was used to determine the relationship of calf gender and pregnancy failure with gestation length, PAG and P4 concentrations. Plasma PAG concentrations at d 32 were lower ($P < 0.05$) in cows that lost pregnancy at d 32–46 and d 46–74 (3.81 vs. 4.83 ± 0.39 ng/mL, respectively) than cows that maintained pregnancy to term (8.64 ± 0.39 ng/mL). Also, there were no difference between cows that retained pregnancy to term and cows that lost pregnancy after d 74 (9.8 ± 0.39 ng/mL). Plasma PAGs were negatively correlated ($P < 0.05$) with parity and age, and positively correlated ($P < 0.05$) with average milk yield. Plasma P4 concentrations at d 32 were lower in cows that lost pregnancy (4.82 ± 0.24 ng/mL) than those that maintained it to term (6.84 ± 0.24 ng/mL). Plasma P4 concentrations were negatively correlated ($P < 0.05$) with parity and age. No correlations were detected between PAG or P4 concentrations and calf gender or gestation length. These observations are consistent with the concept that placental and luteal insufficiencies may be detected in cattle before pregnancy failure. Continued analysis of this data set will explore if peri- and postpartum illnesses affects PAG, P4 and the risk of pregnancy failure in dairy cattle.

Key Words: PAG, dairy, fertility

TH350 Assessment of systematic breeding programs: A comparison between AI after estrus detection and timed AI in lactating dairy cows. A. B. Nascimento*[1], A. H. Souza[2], G. Pontes[1], M. C. Wiltbank[2], and R. Sartori[1], *[1]University of São Paulo, Piracicaba, São Paulo, Brazil, [2]University of Wisconsin, Madison.*

Reproductive management strategies on dairy farms are highly variable with AI either occurring after a fixed time protocol to synchronize ovulation (TAI) or following estrus detection (ED). The first objective of this study was to compare pregnancies per AI (P/AI) at first service in herds using TAI in most of the cows (>80% TAI) versus herds using primarily ED and AI (<20% TAI). The second objective was to evaluate the effect of kg/lactation on P/AI in cows bred after ED or by TAI. Six hundred and 48 herds totaling 83,771 cows were divided into herds using < 20% of TAI and submitted to ED (ED = 25,416) and herds using > 80% of TAI and submitted to TAI (TAI = 58,355). Both categories were divided into 5 levels of milk yield, showed in kg/lactation (P1: 9,072–11,340; P2: 11,341–13,608; P3: 13,609–15,876; P4: 15,877–18,144; and P5: > 18,145). Statistical analyses were performed with logistic regression by PROC GLIMMIX of SAS. There was no difference in overall P/AI at first service between ED (35.4%) and TAI (36.1%). For all cows there was a negative correlation between milk production and P/AI ($r = -0.36$; $P < 0.0001$). Surprisingly, there was no interaction between milk production and AI strategy ($P = 0.85$) as demonstrated by P/AI in ED vs. TAI for P1 (38.0 vs. 37.0%), P2 (37.2 vs. 38.0%), P3 (34.2 vs. 36.0%), P4 (31.3 vs. 34.0%), and P5 (30.5

vs. 29.0%). No advantage of TAI on conception rates over ED-AI was observed, likely due to the great variation in synchronization protocols used in these large herds. In addition, it appears that in this large group of herds, milk production was negatively correlated with P/AI in both cows bred to ED or bred by TAI. Supported by FAPESP, CAPES, and CNPq of Brazil.

Key Words: estrus, TAI, conception rate

TH351 Reproductive outcomes of timed AI or transfer of in vivo- or in vitro-produced vitrified embryos in beef cattle. R. Sartori*[1], A. B. Prata[1], R. S. Surjus[1], A. V. Pires[1], M. C. C. Mattos[2], A. C. Basso[2], J. H. F. Pontes[2], J. R. S. Gonçalves[3], L. G. Lima[3], and T. S. Aguiar[1], *[1]University of São Paulo, Piracicaba, SP, Brazil, [2]In Vitro Brasil Ltda, Mogi Mirim, SP, Brazil, [3]Hildergard G. V. Pritzelwitz Experimental Station, Londrina, PR, Brazil.*

The aim was to evaluate conception rates and pregnancy losses in beef cattle bred by timed AI or that served as embryo recipients. All Nelore (*Bos indicus*) cows (in calf or not) were synchronized with the same protocol within a 3-mo period. On Day 0, cows received i.m. Two mg estradiol benzoate and an intravaginal progesterone device. On Day 8, the device was removed and cows received i.m. treatments of 0.150 mg sodium cloprostenol, 300 IU eCG and 0.6 mg estradiol cypionate. For AI, 346 cows were bred on Day 10 using frozen/thawed semen of 5 bulls. For embryo transfer, cattle received in vivo- (n = 274) or in vitro-produced (n = 573) vitrified embryos on Days 16 to 18 of the protocol after confirming the presence of a corpus luteum. The same groups of cows were used for all treatments. Transfers of in vivo- and in vitro-produced embryos, but not TAI were concurrent, and there were 2 time-periods for AI or ET for each treatment group. Pregnancy diagnosis was performed 30 and 60 d after ovulation by transrectal ultrasound. For in vitro and in vivo embryo production, there were 4 sessions of superovulation or ovum pickup, respectively, 42 d apart in 33 Nelore cows. In vitro embryo production was done using 6 bulls and for in vivo production, 8 bulls were used. There were at least 3 bulls overlapping among AI and ET groups. Grade 1 embryos (IETS) were vitrified using the Cryotop method. Data were analyzed by Chi-squared and are presented below. In conclusion, although having inferior reproductive outcomes as compared with TAI, vitrified embryos produced with oocytes from Zebu cows had promising results as seen by acceptable conception rates and pregnancy losses. Supported by FAPESP, CAPES, and CNPq of Brazil.

Table 1.

	Timed AI	In vivo vitrified	In vitro vitrified
30 d conception, % (no./no.)	50.3 (174/346)[a]	39.4 (108/274)[b]	34.0 (195/573)[b]
60 d conception, % (no./no.)	47.7 (165/346)[a]	35.4 (97/274)[b]	28.6 (164/573)[c]
Embryo/fetal loss (30 to 60 d), % (no./no.)	5.2 (9/174)[b]	10.2 (11/108)[ab]	15.9 (31/195)[a]
Abortion (60 d to calving), % (no./no.)	15.2 (25/165)[a]	6.3 (6/96)[b]	16.5 (27/164)[a]
Peripartum loss, % (no./no.)	2.1 (3/140)[b]	4.4 (4/90)[ab]	9.5 (13/137)[a]

[a,b,c]$P < 0.05$.

Key Words: conception rate, pregnancy loss, vitrification

TH352 Effects of gonadotropin releasing hormone (GnRH) and equine chorionic gonadotropin (eCG) during estrus synchronization and fixed-time artificial insemination of *Bos indicus*-based females on fixed-time artificial insemination and final pregnancy rates. F. R. Gaievski[1], V. R. G. Mercadante[2], G. C. Lamb[2], R. R. Weiss[3], M. A. F. Betiol[3], and L. E. Kozicki*[1], *[1]School of Agricultural Sciences and Veterinary Medicine, Pontifical Catholic University of Parana, Curitiba, PR, Brazil, [2]North Florida Research and Education Center, University of Florida, Marianna, [3]School of Veterinary Medicine, Federal University of Parana, Curitiba, PR, Brazil.*

We determined the effects of GnRH and eCG on fixed-time AI (TAI) and overall pregnancy rates of *Bos indicus* and *Bos indicus* × *Bos taurus* crossbred cows during a 90 d breeding season. A total of 678 females (387 pluriparous and 291 nulliparous females) were assigned randomly (within parity) to 1 of 5 treatments: (1) on d 0 females received a 1.0 mg injection of estradiol benzoate (EB) and insertion of a controlled intravaginal progesterone (P4) releasing device containing 0.558 g of P4, followed on d 9 by P4 device removal and a 0.075 mg injection of prostaglandin $F_{2\alpha}$, a 1.0 mg injection of EB on d 10, and TAI 32 h after EB (TAI1; n = 120); (2) same as TAI1, but inclusion of a 500 µg injection of GnRH at TAI (TAI2; n = 120); (3) same as TAI1, but inclusion of a 400 IU injection of eCG on d 10 (TAI3; n = 120); (4) same as TAI3, but inclusion of a 500 µg injection of GnRH at TAI (TAI4; n = 120); (5) females received no estrus synchronization, but were naturally mated during the 90-d breeding season (natural service; NS; n = 198). Females in the TAI1, TAI2, TAI3, and TAI4 treatments received clean-up bulls for natural service 45 d after TAI, for 45 d. Pregnancy was determined by transrectal ultrasonography on d 45 for TAI pregnancy rates (TAI1, TAI2, TAI3, and TAI4 treatments) and overall pregnancy rates on d 120 after TAI for all treatments. Data was analyzed using the procedure FREQ of SAS (SAS Inst. Inc., Cary, NC). There were no differences (P = 0.61) in pregnancy rates for TAI on d 45 between treatments (TAI1 = 50%; TAI2 = 55%; TAI3 = 58%; TAI4 = 58%). Overall pregnancy rates were greater ($P < 0.001$) for the TAI1, TAI2, TAI3, and TAI4 treatments compared with the NS treatment (85%, 85%, 90%, 93% and 73%, respectively). In conclusion, administration of GnRH or eCG during TAI synchronization did not improve pregnancy rates to TAI; however, exposure of cows to TAI synchronization protocols improved overall pregnancy rates during a 90-d breeding season.

Key Words: artificial insemination, estrus synchronization, *Bos indicus*

TH353 The impact of omission of GnRH at the beginning of 5-d CO-Synch + CIDR program on timed AI pregnancy rate in beef heifers. L. H. Cruppe*[1], G. A. Bridges[2], S. L. Lake[3], R. S. Cipriano[1], F. M. Abreu[1], S. Kruse[2], B. R. Harstine[1], R. Arias[3], R. Raymond[4], W. Kayser[4], M. V. Biehl[1], and M. L. Day[1], *[1]The Ohio State University, Columbus, [2]University of Minnesota, Grand Rapids, [3]University of Wyoming, Laramie, [4]Simplot Livestock Inc, Grand View, ID.*

The objective of this study was to investigate whether the omission of the initial GnRH treatment at the time of CIDR insertion would affect pregnancy rates to timed AI in a 5-d CO-Synch + CIDR program that utilizes a single PGF dose given at CIDR removal. Yearling beef heifers in Ohio (n = 294, Angus × Simmental), Utah (n = 271, Angus × Hereford), Idaho (n = 127, Charolais) and Wyoming (n = 137, Angus) were randomly assigned to either receive 100 µg GnRH (G+, n = 413) or not to receive GnRH (G-, n = 416) at CIDR insertion (d −5). At CIDR removal (d 0 of the experiment), 25 mg PGF2α (PGF; Lutalyse) was administered I.M. to all heifers. All heifers were inseminated by timed AI and given 100 µg GnRH on d 3 (72 h after PGF). In 144 heifers, ovarian ultrasonography was performed on d −5 and 0 to identify CL

present at CIDR insertion and newly formed CL at CIDR withdrawal, respectively. In another subgroup of heifers (n = 269), blood samples for progesterone analysis collected on d 3 (at timed AI) and/or estrus detection were used to assess luteal regression. Pregnancy diagnosis was performed between 32 and 38 d after timed AI. At CIDR withdrawal, presence of a new CL was greater ($P = 0.02$) in the G+ (44.4%, 32/73) than G- (25.3%, 18/71) treatment. In heifers assessed for luteal regression, the G+ (96.3%) and the G- (99.2%) treatments did not differ ($P > 0.1$) in incidence of luteal regression. Timed AI pregnancy rate in the G+ (50.7%) and G- (54.7%) treatments did not differ ($P > 0.1$). In conclusion, omission of the initial GnRH treatment in the 5-d CO-Synch + CIDR program did not influence timed AI pregnancy rate in yearling beef heifers, and the incidence of newly formed CL was only increased by 19.1% with inclusion of the initial GnRH.

Key Words: GnRH, beef heifer, PGF

TH354 The effects of intramuscular or intravenous injections of gonadotropin releasing hormone at fixed-time artificial insemination (TAI) on pregnancies per TAI of *Bos indicus* beef cows. D. Demeterco[1], V. R. G. Mercadante[2], G. C. Lamb[2], F. R. Gaievski[1], B. G. Weiss[1], G. N. Turbay[1], M. S. Segui[1], R. R. Weiss[3], M. A. F. Betiol[3], and L. E. Kozicki*[1], *[1]School of Agricultural Sciences and Veterinary Medicine, Pontifical Catholic University of Parana, Curitiba, PR, Brazil, [2]North Florida Research and Education Center, University of Florida, Marianna, [3]School of Veterinary Medicine, Federal University of Parana, Curitiba, PR, Brazil.*

Our objective was to evaluate the effects of an intramuscular or intravenous administration of gonadotropin releasing hormone (GnRH) at fixed-time AI (TAI) on pregnancies per TAI of crossbred *Bos indicus* beef cows (Nelore × Tabapuã). Pluriparous cows (n = 120) were estrous synchronized as follows: on d 0 cows received a 2.0 mg injection of estradiol benzoate (EB) and insertion of a controlled intravaginal progesterone releasing device containing 0.558 g of progesterone, followed on d 8 by removal of the progesterone device simultaneous with a 0.15 mg injection of prostaglandin $F_{2\alpha}$ (PGF), a 1.0 mg injection of EB, and 400 IU injection of equine chorionic gonadotropin. At 54 h after PGF all cows received a fixed-time AI and a 0.084 mg injection of GnRH (buserelin acetate) administered either via the vena caudalis (n = 60), or via the longissimus dorsi (n = 60). All cows were inseminated with the same AI sire and by a single experienced AI technician. Pregnancy was determined by transrectal ultrasonography on d 60 after AI. Data was analyzed by Chi Square analysis using the procedure FREQ of SAS (SAS Inst. Inc., Cary, NC). Cows receiving the intravenous administration of GnRH had greater ($P = 0.04$) pregnancies per TAI than cows receiving the intramuscular injection of GnRH at the time of AI (65% vs. 46.6, respectively). We concluded that intravenous administration of GnRH at the time of AI improved pregnancies per TAI of crossbred *Bos indicus* beef cows submitted to TAI.

Key Words: gonadotropin-releasing hormone, artificial insemination, intravenous

TH355 Timing of artificial insemination in a 7-d P4-E2 estrous synchronization program in *Bos indicus* postpartum cows. M. V. C. Ferraz Junior[1], A. V. Pires[2], M. V. Biehl*[1], E. M. Ferreira[2], D. D. Nepomuceno[2], V. N. Gouvea[1], R. Sartori[2], J. R. S. Goncalves[3], L. H. Cruppe[4], and M. L. Day[4], *[1]University of Sao Paulo, Pirassununga, SP, Brazil, [2]University of Sao Paulo, Piracicaba, SP, Brazil, [3]Experimental Station Hildegard Georgina Von Pritzelwitz, Londrina, PR, Brazil, [4]The Ohio State University, Columbus.*

The aim of this study was to compare the effect of altering the moment of timed AI (TAI) based upon occurrence of estrus for a 7-d P4-E2 estrus synchronization protocol in Nellore cows. Two hundred and 60 postpartum cows (multiparous, n = 201; primiparous, n = 59; body condition score 2.7 ± 0.01; BW, 434 kg ± 3.8) that were 40 ± 1.2 d postpartum (d 0) were used. On d 0, all cows received 2 mg of estradiol benzoate (Estrogin) and a new CIDR. At CIDR removal on d 7 (designated as h 0), 25 mg of PGF (Lutalyse), 0.6 mg of estradiol cipionate (ECP) and 300 IU of eCG (Folligon) were administered in all cows. Cows were either all inseminated by timed AI (TAI) at h 55 (55h, n = 130) or cows detected in estrus by h 55 were TAI at h 55, with the remainder of cows not detected in estrus by h 55 receiving TAI at h 72 (55/72h, n = 130). Estrus detection was performed using Estrotect patches to either h 55 (55h treatment) or h 72 (55/72h treatment). Blood samples collected 11 d after CIDR removal were analyzed for P4 concentration to confirm ovulation during synchronization. Data were analyzed using GLIMMIX procedures of SAS. Estrus detection rate was greater ($P < 0.01$) for the 55/72h (84.6%) than 55h (67.7%) treatment; probably due to the longer period of estrus detection. Ovulation rate (55/72h, 89.2%; 55h, 91.5%) and TAI pregnancy rate (55/72h, 59.2%; 55h, 55.4%) did not differ ($P > 0.1$) between treatments. No interaction between treatment and parity was detected. In a secondary analysis across treatments, estrus detection rate was greater ($P < 0.01$) in multiparous (81.1%) than primiparous (59.3%) cows. Accordingly, ovulation rate was greater ($P = 0.02$) in multiparous (93.5%) than primiparous (79.7%) cows. In contrast, TAI pregnancy rate did not differ ($P > 0.1$) between multiparous (58.2%) and primiparous (54.2%) cows. In conclusion, the 55/72h approach, in which TAI in cows not detected in estrus within 55 h was postponed until h 72 did not increase TAI pregnancy rate compared with TAI of all cows at h 55.

Key Words: postpartum beef cow, E2-P4 protocol, timing of AI

TH356 Improving embryo recovery from superovulated Holstein dairy cattle: Evaluation of reflushing 30 minutes after the initial flush on embryo recovery. R. W. Bender*, K. S. Hackbart, P. D. Carvalho, A. R. Dresch, L. M. Vieira, M. C. Amundson, G. B. Sandoval, A. H. Souza, J. N. Guenther, and M. C. Wiltbank, *University of Wisconsin-Madison, Madison.*

Non-surgical embryo recovery techniques revolutionized superovulation after introduction in 1976; however, suboptimal embryo recovery continues to be a problem. In experiment 1, superovulated Holstein cows were flushed (n = 156 flushings from 32 lactating and 17 dry cows) with a silicone 2-way catheter in each horn individually using a liter of flush media per horn (SF). Following the initial uterine horn flush, the catheter was moved back to the cervix and flush media was placed in the uterus. After 30 min, cows had their entire uterus reflushed with one liter of flush media (DF). Four experienced technicians performed all flushes and one experienced technician performed all searching of structures. Superovulatory response (CL number using ultrasound) averaged 17.3. A total of 8.4 structures (48.6%; fertilized and unfertilized oocytes) were recovered during initial flushing vs. 9.9 (57.2%) after flush/reflush. Thus, reflush increased yield of structures by 17.9% (1.5 structures). Experiment 2 evaluated whether reflush could be done immediately by doing a full uterine reflush immediately after initial horn flushing, followed by a second reflush 30 min later (n = 14). Superovulation averaged 19.6 CL with 10.1 structures recovered after initial flush (51.5%), with immediate reflush yielding 1.1 structures (5.6%) and later reflush 2.5 structures (12.8%). Combining results from both experiments, reflushing increased ($P < 0.0001$) embryo yield 18.9% with no interaction ($P = 0.36$) between superovulatory response and flush technique on recovery

(<10CL: 38.4% vs. 42.6%; 10 – 20CL: 54.9% vs. 67.8; > 20CL: 47.7% vs. 54.9%, flush vs. flush/reflush, respectively). Surprisingly, cows with low (<50%) structure recovery from the initial horn flush had lower embryo recovery from reflush than cows with high (>50%) initial embryo recovery (0.9 vs. 1.9 additional structures). In conclusion, the reflushing technique increased embryo recovery and could be of value, particularly in donor cows of high genetic merit and cows that have high structure recovery during the initial horn flushing.

Key Words: superovulation, dairy, embryo

TH357 Identifying and resynchronizing open cows and heifers 21 d after AI using CIDR inserts, ultrasonography, and GnRH in dairy cattle. L. Ibarbia, J. H. Bittar, R. Daetz, J. E. Santos, C. A. Risco, and K. N. Galvão*, *University of Florida, Gainesville.*

Reinsemination interval (RINT) is at least 42 d when diagnosing pregnancy on d 32 after AI and using timed AI for reinsemination. Objective was to decrease RI when dairy cows or heifers are inseminated using timed AI programs. Holsteins heifers (n = 153) and cows (n = 204) from one herd were randomly allocated into one of 2 groups: 21dRES (n = 77 heifers and 102 cows) had a CIDR inserted on d 13 and removed on d 20 after AI, and ovaries scanned by ultrasonography. Animals found not to have a CL and to have a follicle ≥ 12 (heifers) or ≥ 15mm (cows) received GnRH and TAI in the morning of d 21. Pregnancy diagnosis was performed on d 32, and nonpregnant 21dRES animals not reinseminated (RINS) on d 21 were started on Ovsynch-56 immediately (cows) or on the 5d-CoSynch (heifers) on d 34. Control animals were RINS after nonpregnancy diagnosis using Ovsynch-56 (cows) or 5d-CoSynch (heifers) starting on d 32 or d 34, respectively. Data were analyzed using PROC GLIMMIX in SAS. Conception rate (CR) for initial AI was similar for 21dRes and Control in heifers (49.4 vs. 51.3%) and cows (32.4 vs. 29.4%). Of the open animals, 59.0% (23/39) of heifers and 37.7% (26/69) of cows in 21dRES were RINS on d 21. Only one pregnant heifer was inaccurately diagnosed open on d 20 and RINS on d 21 (97.4% Specificity). RINT was decreased by 12.2 d (30.1 vs. 42.3 d; $P < 0.001$) in heifers and by 8.7 d (34.6 vs. 43.3; $P < 0.001$) in cows. Overall resynchronized CR was increased for 21dRES heifers compared with Control (57.5 vs. 32.4%; $P = 0.03$) because heifers RINS on d 21 had similar CR compared with Control RINS using CoSynch (43.5 vs. 32.4%; $P = 0.39$), but CR was higher for 21dRES RINS using CoSynch than Control (76.5 vs. 32.4%; $P = 0.003$). Overall resynchronized CR for cows was similar for 21dRES and Controls (30.0 vs. 30.0%; $P = 1.0$). CR was similar ($P > 0.7$) for 21dRES cows RINS on d 21 (26.9%) or using Ovsynch (35.7%) compared with Controls (30%). In conclusion, 21dRES resulted in decreased reinsemination interval, similar CR for initial AI and AI on d 21, and increased (heifers) or similar (cows) overall reinsemination CR compared with Control.

Key Words: reinsemination, timed AI, dairy cattle

TH358 Effects of heat stress and insulin on hepatic progesterone catabolic enzymes cytochrome P450 2C and 3A in lactating cows. V. L. McCracken*[1], G. Xie[1], S. E. Deaver[1], L. H. Baumgard[2], R. P. Rhoads[1], and M. L. Rhoads[1], *[1]Virginia Polytechnic Institute and State University, Blacksburg, [2]Iowa State University, Ames.*

Two experiments were performed to determine the effects of heat stress (HS) and insulin on hepatic mRNA abundance of enzymes responsible for catabolizing progesterone (cytochrome P450 2C and 3A [CYP2C and CYP3A]). To distinguish the direct effects of HS from the inherent effects of decreased DMI, a group of contemporaries was pair-fed (PF) to match the intake of the HS cows during both experiments. In the first experiment, multiparous lactating Holstein cows (n = 12) housed in climate-controlled chambers were subjected to 2 experimental periods (P1 and P2): 1) thermoneutral (TN) conditions (18°C, 20% humidity) with ad libitum intake (TN and well-fed [WF]) for 9d; and 2) either HS conditions (cyclical temperature 31.1–38.9°C, 20% humidity) fed for ad libitum intake (n = 6), or TN conditions, pair-fed (PF) to match the intake of HS animal (n = 6) for 9d. Liver samples were obtained at the end of each period and relative mRNA abundance was measured by real-time RT-PCR. In the second experiment, multiparous lactating Holstein cows (n = 12) were housed and fed as described for the first experiment. Liver samples were obtained immediately before and after an insulin tolerance test on the last day of each period. Gene expression data were analyzed using the Mixed Procedure of SAS. Heat stress caused the hepatic expression of CYP2C to decrease during both experiments ($P = 0.07$ and $P < 0.01$ for experiments 1 and 2, respectively). The relative abundance of CYP3A was not affected by environmental conditions in the first experiment but was reduced by HS in the second experiment ($P < 0.01$). Interestingly, during experiment 2 hepatic CYP3A expression also decreased during PF ($P < 0.05$). There were no effects of insulin treatment nor were there interactions between treatment groups (TN/HS or WF/PF) and insulin. Taken together, these results indicate that HS may affect progesterone catabolism by altering the hepatic expression of CYP2C and CYP3A.

Key Words: progesterone, heat stress, insulin