activity. Interestingly, ingestion of combinations of these bioactives can enhance bioavailability lower down in the colon.

Key Words: swine, natural, sustainable, bioactive, gut health

PHYSIOLOGY AND ENDOCRINOLOGY

1039 WS Influence of sampling location and pregnancy on composition of the microbiome associated with the reproductive tract of the ewe.

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The objective of this study was to investigate the microbiome of the vagina, uterus, and embryo and determine the effects of pregnancy status and a maternal pregnancy recognition antagonist treatment. We hypothesized that location, pregnancy status, and maternal pregnancy recognition antagonist treatment would result in significant differences in the bacterial microbiome of the reproductive tract in sheep. Mini osmotic pumps were placed surgically into the uterus and loaded with control (PBS, $n = 9$) or treatment (AMD3100, $n = 7$). AMD3100 is an antagonist for maternal pregnancy recognition. Samples were collected for microbiome analysis from the vagina, uterus, and embryo. AMD3100 and PBS had no effect on microbiome composition ($P > 0.98$). Sampling location had the greatest effect on bacterial population ($P > 0.01$). *Firmicutes*, *Proteobacteria*, and *Actinobacteria* were the most predominant phyla ($P < 0.01$) present in the vagina while *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were present in the uterus ($P < 0.01$). The genus of bacteria present in the uterus and vagina supported the phylum data. *Corynebacterium* was more prevalent than *Finegoldia* in the vagina ($P < 0.01$), while the prevailing genus in the uterus was *Bradyrhizobium* ($P < 0.01$). The pregnancy status of ewes did not differ by phylum; however, the genus *Finegoldia* was greatest in nonpregnant ewes ($P < 0.01$). Treatment effects were not observed on embryo microbiome phylum ($P < 0.90$) or genus ($P < 0.88$). Results show further research is needed to understand the relationship between the reproductive tract microbiome and ewe fertility.

Key Words: metagenome, sheep, uterus, vagina

1040 Use of doppler ultrasound and infrared thermography to evaluate scrotal insulation in Braford bulls.

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The objective of this study was to evaluate the flow dynamics of scrotal surface temperature (SST) by infrared thermography in Braford bulls submitted to scrotal insulation. In addition, bulls were also evaluated for velocity parameters (V), pulsatility index (PI), and resistance index (RI) using Doppler ultrasound. All procedures were approved by the Ethical Committee for Care and Use of Experimental Animals (Project 19656/2014/58, CEUA/UEL). All animals had a breeding soundness examination at the beginning of the experiment. Eight Braford bulls were used at the age of 18 mo and randomly divided into four different groups, as follows: the control group not subjected to insulation (CON; $n = 2$), scrotal insulated bulls for 72 h (G72, $n = 2$), scrotal insulated bulls for 96 h (G96, $n = 2$), and scrotal insulated bulls for 120 h (G120, $n = 2$). Infrared thermography and Doppler data were collected at four different periods: after removal of scrotal insulation (M0), 10 min after removal of scrotal insulation (M10), 30 min after removal of scrotal insulation (M30), and 60 min after removal of scrotal insulation (M60). Data were analyzed using ANOVA, *t* test (paired), and Pearson correlation with a significance level of 5%. No differences were observed between insulated treated groups. Rectal temperature (38.5 ± 0.4) was higher compared to scrotal surface (32.7 ± 0.8; $P < 0.05$). Scrotal insulated animals showed higher testicular temperature at M0 (33.0 ± 0.7) compared to M10, M30, and M60 periods (30.2 ± 1.3, 31.6 ± 1.5, 30.6 ± 1.0, respectively; $P < 0.05$). We observed no difference in PI and RI indexes between evaluation periods after scrotal insulation. However, blood flow velocity (cm/sec) showed differences between M10 (17.1 ± 4.22) compared to the M0, M30, and M60 periods (12.5 ± 5.1, 14.3 ± 4.5, 14.3 ± 2.9, respectively; $P < 0.05$). A positive correlation (93.1%) was observed between PI and RI ($P < 0.05$) variables. The scrotal insulation changes the temperature and the blood flow velocity; however, after 60 min of insulation, these parameters are already reestablished. We conclude that Doppler ultrasound can be used to evaluate scrotal blood flow variations during scrotal heat stress induction in Braford bulls.

Key Words: Doppler ultrasonography, infrared thermography, scrotal insulation

1041 Diurnal vaginal temperature cycles of Senepol and crossbred beef heifers with different hair coat types and colors under tropical conditions.

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This study evaluated the influence of hair coat type and color on vaginal temperature (VT) regulation of beef heifers. The VT was recorded in five slick haired, red colored Senepol (SENEPOL); six slick haired, light colored crossbred (SLICK); and four wild-type long haired, light colored crossbred (REGULAR) heifers, every 5 min for four consecutive days. Crossbred heifers were obtained from Charbray × Senepol × Charolais crosses. Air temperature and relative humidity were recorded in synchrony with VT, and the thermal humidity index (THI) was determined. Heifers were kept in a large paddock with access to natural shade. After averaged by hour, data were analyzed by the GLIMMIX and CORR procedures of SAS. To study the possible lag time in the relationship between the THI and the TV, the correlations were performed taking into consideration the THI recorded previous to the VT in 1-hour intervals (from 1 to 24 h earlier). Hair type-color interacted with the time of day to affect VT, with REGULAR heifers presenting greater VT values than SLICK and SENEPOL from 2200 to 2400 h (39.33 ± 0.03, 38.84 ± 0.01, and 38.74 ± 0.02°C, respectively). During the remaining daily time period (0100 to 2100 h), similar VT values were observed in the REGULAR, SLICK, and SENEPOL heifers (38.75 ± 0.01, 38.62 ± 0.01, and 38.51 ± 0.01°C, respectively; $P > 0.05$). The greatest positive correlations between THI and VT were obtained when evaluating the THI value from 7 h earlier in SENEPOL ($r = 0.25$; $P < 0.0001$) and SLICK ($r = 0.30$; $P < 0.0001$) and from 8 h earlier in REGULAR ($r = 0.32$; $P < 0.0001$) heifers. Regardless of coat color, the slick hair phenotype allowed heifers to minimize their daily increase in body temperature in comparison with their long haired counterparts. We suggest introducing genes for the slick phenotype of the Senepol into other Puerto Rican beef breeds as a means of reducing the negative impact of heat stress.

Key Words: coat color, slick hair, vaginal temperature

1042 Associations between the environmental conditions and vaginal temperature in wild-type and slick-haired Puerto Rican Holstein cows.

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The physical barrier created by the hair coat between the skin and the environment has been reported to reduce heat dissipation in wild-type-haired (WT) *Bos taurus* cattle exposed to hot and humid weather. However, slick-haired (SLICK) cattle have demonstrated the ability to maintain lower body temperatures under similar conditions. Thus, the present study aimed to evaluate if the air temperature (AT), relative humidity (RH), thermal humidity index (THI), solar radiation (SR), wind speed (WS), and gust speed (GS) have a different relationship with vaginal temperature (VT) in Holstein cows with different hair coat types during the summer in Puerto Rico. Twenty-four lactating cows [11 WT; 176.31 ± 32.65 d in milk (DIM); 2.06 ± 1.44 lactations and 13 SLICK; 175.62 ± 42.78 DIM; 2.19 ± 0.96 lactations] were evaluated. The WT and SLICK classifications were confirmed in previous genotyping studies. Data loggers (Onset Computer Corporation, Bourne, MA, USA) recorded environmental variables as well as VT values in synchrony every 5 min for 7 consecutive days. After averaged by hour, data were analyzed by the GLIMMIX procedure of SAS. The CORR procedure of SAS was used to analyze the data collected every 5 min. Time and hair type interacted ($P = 0.0026$) to affect VT. From 1800 to 0700 h and from 0900 to 1600 h, the WT cows presented, on average, 0.31°C greater VT values than their SLICK counterparts ($P = 0.0032$). During the 0800 h ($P = 0.0584$) and 1700 h ($P = 0.0619$) VT values tended to be, on average, 0.20°C greater in WT than in SLICK cows. In WT cows VT was correlated with AT ($r = 0.43$; $P < 0.0001$), RH ($r = –0.38$; $P < 0.0001$), THI ($r = 0.45$; $P < 0.0001$), SR ($r = 0.16$; $P < 0.0001$), WS ($r = 0.38$; $P < 0.0001$), and GS ($r = 0.38$; $P < 0.0001$). In the SLICK group, greater correlation coefficients were obtained between VT and AT ($r = 0.50$; $P < 0.0001$), RH ($r = -0.44$; $P < 0.0001$), THI ($r = 0.53$; $P < 0.0001$), SR ($r = 0.24$; $P < 0.0001$), WS ($r = 0.46$; $P < 0.0001$), and GS ($r = 0.46$; $P < 0.0001$). Our results suggest that the slick-haired phenotype allows cows to have a more direct relationship between their skin and the environment, providing for greater heat dissipation and a lower body temperature.

Key Words: heat stress, slick-haired, vaginal temperature

1043 Impact of heat stress and metabolic endotoxemia on porcine ovarian function. M. J. Dickson*, K. L. Bidne, B. J. Hale, C. L. Hager, J. T. Seibert, L. H. Baumgard, J. W. Ross, and A. F. Keating, *Iowa State University, Ames.*

Heat stress (HS) results from an imbalance of thermal energy, and economic losses due to HS cost the U.S. swine industry approximately $900 million annually. HS negatively influences a variety of parameters affecting reproduction: spontaneous abortion, longer wean-to-estrus interval, delayed puberty, reduced litter size, and total number born, all of which culminate in seasonal infertility. Additionally, HS compromises intestinal integrity ultimately leading to metabolic endotoxemia (ME) and increased systemic lipopolysaccharide (LPS), an endotoxin originating from the cell wall of gram-negative bacteria. Toll-like receptor 4 (TLR4) is a membrane bound receptor protein that binds LPS, initiating a signaling cascade that involves phosphorylated nuclear factor kappa B (pNFκB) and pro-inflammatory cytokine release. Acyloxyacyl hydrolase (AOAH) cleaves the lipid A moiety from LPS and is thereby involved in LPS detoxification. We hypothesized that ME contributes to seasonal infertility in swine. The study objectives were to characterize the impact of HS and ME on ovarian function. Twelve post-pubertal gilts (126.0 ± 21.6 kg) were synchronized for 14 d by orally administering Matrix® to ensure that gilts were heat-stressed during the follicular phase of the estrous cycle. Immediately after Matrix® withdrawal, gilts were split in two groups ($n = 6$) and exposed to cyclical HS or thermal neutral (TN) conditions for 5 d and then sacrificed. Gilts were exposed to either constant TN conditions (20.3 ± 0.5°C) or cyclical HS (25.4– 31.9°C) to imitate a diurnal heat load pattern. Ovaries were collected at the end of the experimental period and TLR4, pNFκB, and AOAH protein abundance were quantified in whole ovarian lysate. Relative to TN ovaries, HS increased ($P < 0.05$) TLR4 (16%) and pNFκB (11%) protein abundance. There was no difference in AOAH abundance ($P > 0.05$) between TN and HS ovaries. This data suggests that the ovary is responsive to ME and that HS can induce a pro-inflammatory environment in the ovary, which could contribute to compromised fecundity in heat-stressed swine. This work was supported by the National Pork Board.

Key Words: heat stress, immune function, ovary

1044 Heat stress induces distinct lipidomic profile in differentiating porcine adipocytes. H. Qu[1] and K. M. Ajuwon*[2], *[1]Purdue University, West Lafayette, IN, [2]Department of Animal Sciences, Purdue University, West Lafayette, IN.*

Heat stress results in enhanced lipid deposition in pigs. However, the effects of heat stress (HS) on adipocyte lipidome and metabolome are largely unknown. Understanding the effects of heat stress on the lipid profile will increase understanding of heat stress sensing and signaling mechanisms. To study this, we applied a combination of liquid chromatography-mass spectrometry (LC-MS) based metabolomic and lipidomic profiling approaches to identify and characterize the lipid classes in differentiating pig adipocytes. Porcine preadipocyte (stromovascular cells) were differentiated either under control (37°C) or HS (41.5°C) temperature conditions for 9 d. HS increased triglycerides and decreased monoacylglycerols ($P < 0.05$) accumulation. HS also increased concentrations of glycerophosphocholines, glycerophosphoserines, glycerophosphoglycerols, and glycerophosphoinositols than in control ($P < 0.05$). The specific lipidomic signatures in HS indicates that metabolic pathways centered around diacylglycerol (DAG) metabolism may be impacted by HS in pig adipocytes, perhaps as part of an adaptive mechanism. The observed changes in phospholipid composition may help to regulate cellular metabolism, membrane characteristics, and signal transduction pathways for enhanced adaptation to HS. Overall lipidomic analysis revealed that HS induces a unique lipidomic profile in adipocytes.

Key Words: pig adipocytes lipidomics LC-MS

1045 Impact of temperature fluctuations in cooled-fresh semen on fertility of lactating dairy cows. A. H. Souza*[1], H. J. Bessoff[2], and E. Danzeisen[3], *[1]Ceva Animal Health, Libourne, France, [2]Dairy Management Solutions, Tulare, CA, [3]Global AG Alliance, Tulare, CA.*

The objective of this study was to evaluate whether deviations from ideal semen storage temperature of cooled-fresh semen could affect conception results (CR) in lactating dairy cows. A temperature recording system (LogTag® analyzer, Trix8 data logger model) was placed in the semen shipping-container from its initial shipping to the dairy (located in the Central California Valley) and continuously until breeding time. Temperature was recorded for breedings that took place from June 2015 to December 2015 at every 30 s interval with a 0.01°C of accuracy. Ideal storage temperature for fresh semen was assumed to be from 1.7 to 7.2°C. Then, the cumulative amount of time within the 24 h before AI in which semen temperature deviated to below the lower limit and/or above the high temperature limit was utilized in the Glimmix (SAS 9.3) logistic model to evaluate the impact of temperature fluctuations on

CR. A total of 2306 AI records were available for CR analysis. Variables considered in the logistic regression model were herd, parity number of the cow, days in milk at AI, year-month of AI, service sire, AI technician, temperature deviations from below and above ideal limits, and cow-within herd, which was included in the model as a random variable. As expected, most deviations from ideal storage temperature were due to above threshold temperature faults; and those were more common in summer months, particularly during months of June and August, occurring more commonly from 8AM to 1PM. Interestingly, semen storage deviations to above temperature limits appeared to have less impact ($P > 0.10$) on CR than when temperature deviated to below the lower limits ($P = 0.03$). As a result, CR was 36.2% when the temperature deviated less than 5% of the daily time above the temperature threshold limit and had a nonsignificant minor decrease to 35.1% when temperature deviated over 20% of the daily time above the upper temperature limits. In contrast, CR significantly decreased from 35.8% when semen deviated less than 5% of the time toward below lower temperature limits, to 31.3% when temperature deviated more than 20% of the daily time to below the lower temperature limit. In conclusion, it appears that exposing cooled-fresh semen to colder temperatures below 1.7°C was more detrimental to the fertility of dairy cows.

Key Words: fresh semen, storage temperature, fertility

1046 Effects of a 48 h feed withdrawal on intraperitoneal core body temperature in growing pigs. J. S. Johnson*[1], N. M. Chapel[2], and C. J. Byrd[2], *[1]USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN, [2]Purdue University, West Lafayette, IN.*

In response to increasing ambient temperatures, pigs often reduce their feed intake (FI) to decrease metabolic heat production and maintain euthermia. Although the effects of reduced FI on swine body composition, metabolism, growth rate, and reproductive parameters are well-documented, little is known regarding the direct effects of feed withdrawal on core body temperature response. Therefore, the study objective was to determine the effects of a 48 h feed withdrawal on the intraperitoneal core body temperature response in growing pigs. Eight barrows (35.9 ± 1.9 kg BW) were housed in TN conditions (22.30 ± 0.22°C) and exposed to four 24 h periods: 1) PT (pre-treatment ad libitum feeding), 2) FW1 (1–24 h feed withdrawal), 3) FW2 (25–48 h feed withdrawal), and 4) RF (ad libitum re-feeding). Barrows were used in an effort to reduce the body temperature variability associated with gonadal steroid production. Intraperitoneal core body temperature (T_{core}) was recorded in 10 min intervals using implanted Thermochron temperature recorders. Data were analyzed using the PROC MIXED procedure in SAS 9.4. Overall, T_{core} was reduced ($P < 0.01$) during FW1 (40.30°C) and FW2 (40.28°C) compared to PT (40.59°C) and RF (40.60°C), but no differences were observed between the FW1 and FW2 or the PT and RF periods. Minimum T_{core} was reduced ($P < 0.01$) during FW1 (39.86°C) and FW2 (39.81°C) compared to PT (40.34°C) and RF (40.28°C), but no differences were observed comparing the FW1 and FW2 or the PT and RF periods. No maximum T_{core} period differences ($P = 0.32$) were observed. During FW1, a linear reduction ($P < 0.01$; −0.03°C/h) from maximum (h1) to minimum (h21) T_{core} was observed. Between h 24 of the FW2 period and h 1 of the RF period, T_{core} was linearly increased ($P < 0.01$) by 0.66°C. Feed withdrawal increased ($P < 0.01$) the T_{core} variance and range during FW1 (0.07 and 0.96°C, respectively) and FW2 (0.07 and 0.90°C, respectively) compared to the PT period (0.02 and 0.50°C, respectively) and during the FW1 compared to the RF period (0.02 and 0.69°C, respectively). No T_{core} variance or range differences were detected between the PT and RF periods or the FW1 and RF periods. In summary, a 48 h feed withdrawal directly reduced T_{core} in growing pigs, and this T_{core} reduction recovered within the first hour of re-feeding.

Key Words: pigs, feed withdrawal, core body temperature

1047 The effect of exercise on heat tolerance and first lactation in pregnant Holstein heifers. J. Johnson*, P. L. Steichen, and T. G. Rozell, *Kansas State University, Manhattan.*

A primary source of stress for dairy cattle is associated with the environment, particularly heat, and therefore a considerable amount of research has been done in an attempt to find ways of reducing heat stress. Exercise improves heat regulation in humans and horses; thus, the objectives of this study were to determine if an exercise regimen could improve thermo-tolerance and subsequent milk production during the hot part of the summer in Kansas. Pregnant Holstein heifers ($n = 24$) were randomly assigned to 2 treatment groups: exercise (EX; $n = 12$), and exercise-control (EC; $n = 12$; walked with exercise heifers to exerciser but held in a holding pen). An exercise regimen was implemented through May and June, 4 d per wk in the afternoon for approximately 30–45 min using a motorized 8-panel walker. Data were collected on fitness test d 0, 28, and 56 of the experiment in which heifers were exercised for 23 min, 10 of which were spent at a greater intensity (5.63 KPH). Intra-vaginal temperature, skin temperature, respiration rates, and heart rate were recorded. Weekly measurements of skin temperature, respiration rates, and rectal temperatures were also recorded and post-parturition milk production and milk components were determined. All data were analyzed using PROC MIXED. Respiration rates and heart rates were not affected by exercise treatment on fitness test days or during weekly measurements ($P > 0.10$). Time spent in body temperature zone 3 (> 40.0°C) during the 23-min fitness test and 1 h following fitness test tended to be greater for EC than EX (87% vs. 76%; $P < 0.10$). Average

body temperature of the hour following fitness tests was significantly less in EX than EC (40.48°C vs. 40.83°C; $P < 0.05$) on d 28. On fitness test Day 28, EX heifers tended to have reduced skin temperatures at the thurl post-exercise compared with EC ($P < 0.10$). Exercised heifers had reduced skin temperatures of the cheek, withers, and thurl compared with EC during week 7 ($P < 0.05$). Exercise resulted in greater milk protein % and solids-not-fat % ($P < 0.05$) compared with EC, but there was no difference in monthly milk production in the first 150 d of lactation ($P > 0.10$). These results indicate that exercise in pregnant dairy heifers may improve heat tolerance, and improve milk quality during first lactation.

Key Words: exercise, heat tolerance, milk quality

1048 Effect of exercise on ovarian function in cycling gilts.

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Exercise can alter reproductive function in the mare. To identify this phenomenon in other species, the effects of daily exercise on ovarian function was evaluated in cycling gilts. A total of 18 gilts (mean age = 225 ± 8.3 d) were treated orally with a synthetic progestin, and subsequently injected with a gonadotropin to synchronize estrous cycles. Gilts were then trained to follow a target and run voluntarily along an 80 m track. Thereafter, gilts were randomly assigned to either an exercise or control group. Exercised pigs were worked twice daily for 6 min each period, during the last 10 d of the estrous cycle. Each exercise period consisted of an average distance of 0.25 km at an average speed of 6.0 km/h. Rectal temperatures increased from values of 38.5 ± 0.3°C at rest to a mean of 38.8 ± 0.4°C immediately after exercise ($P < 0.05$). Respiration rates also increased from 30.8 ± 3.5 to 59.7 ± 12.6 breaths/min ($P < 0.05$). Cortisol was measured in saliva the day before the exercise protocol started and 5 and 9 d later. Cortisol concentrations were higher ($P < 0.05$) in exercised pigs at 5 and 9 d compared to controls. Gilts were slaughtered 2 d after the onset of estrus and reproductive organs were collected. No differences were found among treatments in the total number of follicles, corpus hemorragica, or corpora lutea. Exercised gilts had more medium (18.5 vs. 7.6; $P < 0.01$) and small (24.6 vs. 20.1; $P < 0.05$) sized follicles compared to control gilts. Cumulus-oocyte complexes were aspirated from small follicles (< 2 mm in diameter), medium follicles (3–6 mm) and large follicles (> 6 mm) and classified by quality based on a qualitative scale considering the number of layers of compact cumulus cells and ooplasm homogeneity. However, there were no effects of treatment on oocyte complex quality. Data indicated that exercise of pigs is associated with a stress response and can influence ovarian follicle development.

Keywords: exercise, follicular development, stress

1049 The effect of exogenous glucose infusion on early embryonic development in lactating dairy cows.

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The objective of this study was to examine the effect of glucose infusion in late lactation (276 ± 17.3 d in milk) dairy cows on early embryonic development. Estrous cycles were synchronized using a progesterone ovsynch protocol ($n = 12$ cows). On Day 7 after synchronized estrus, cows were randomly assigned to either intravenous glucose infusion (750 g/d; 78 mL/h of 40% glucose, GLUC) or intravenous saline infusion (78 mL/h of 0.9% saline solution, CTRL) for 7 d ($n = 6$/treatment). In vitro produced Day 7 blastocysts (15 per cow) were transferred into all cows on Day 7 of the estrous cycle at the same time as the infusion commenced. Blood samples were collected at 0600 and 1800 every day for glucose, NEFA, BHB and progesterone determination, and transrectal ultrasound was used to measure corpus luteum (CL) volume every second day. All cows were slaughtered on Day 14. Reproductive tracts were recovered and flushed with phosphate-buffered saline containing 5% FCS. The number and dimensions (length and width) of recovered embryos were recorded. Endometrial tissue was dissected and snap frozen for later determination of mRNA abundance of glucose transporters (GRT3, GTR8, SLC2A1, SLC2A3, SLC35A4 and SLC5A1) using qPCR. There was no effect of treatment on milk yield (14.1 ± 1.3 vs. 14.7 ± 1.3 kg/d) or dry matter intake (15.0 ± 1.6 vs. 14.4 ± 0.8 kg/d). Glucose infusion increased mean (± SE) circulating glucose concentration (4.7 vs. 4.15 ± 0.1 mmol/l, $P < 0.001$) and reduced circulating BHB concentration (0.51 vs. 0.70 ± 0.01; $P < 0.001$); plasma NEFA concentrations were not affected (0.13 vs. 0.14 ± 0.01). Mean circulating progesterone concentration (6.8 vs. 6.8 ± 1.0 ng/ml) and CL volume were not affected by treatment. There were no effects of treatment on either uterine lumen fluid glucose concentration or mRNA abundance of glucose transporters. Embryo development was decreased in the GLUC cows compared with CTRL cows (length: 11.5 vs. 18.3 ± 3.05 mm; width: 1.3 vs. 1.7 ± 0.15 mm; area: 15.0 vs. 28.7 ± 3.5 mm^2, all $P < 0.05$). A greater proportion of embryos recovered from CTRL cows had elongated to ≥ 16mm in length compared with GLUC cows (0.16 vs. 0.51 ± 0.12; $P = 0.07$).

These results indicate that increasing circulating glucose concentration during the period of conceptus elongation before maternal recognition of pregnancy had an adverse effect on early embryonic development.

Key Words: embryo development, endometrium, glucose

1050 Influence of cattle temperament on blood serum fatty acid content. T. Gardner[*1], J. F. Legako[1], N. C. Burdick Sanchez[2], P. R. Broadway[2], J. A. Carroll[2], R. C. Vann[3], *[1]Utah State University, Logan, UT, [2]Livestock Issues Research Unit, USDA-ARS, Lubbock, TX, [3]MAFES-Brown Loam, Mississippi State University, Raymond, MS*

Cattle temperament has been reported to influence blood metabolites. Specifically, temperament was related with increased circulation of serum NEFA, decreased blood urea nitrogen, and reduced insulin sensitivity. Metabolic alterations such as these may impact cattle immune function, performance traits, carcass traits, and meat tenderness. Presently, little work has been performed to determine the impacts of temperament on fatty acid content within blood serum. For this study blood and resulting serum was obtained from Angus-cross steers ($n = 31$; 216 ± 6 kg BW), previously assessed to be Temperamental ($n = 15$) or Calm ($n = 16$). Temperament score was calculated as an average of exit velocity and pen score measured at weaning. Serum fatty acid content (mg/mL) was determined via gas chromatography and flame ionization detection. Serum from Temperamental steers contained greater ($P \leq 0.050$) concentrations of linoleic (18:2 n-6; 2.56 mg/mL), α-linolenic (18:3 n-3; 0.34 mg/mL), dihomo-γ-linolenic (20:3 n-6; 0.12 mg/mL), and eicosapentanoic acid (20:5 n-3; 0.26 mg/mL) compared with Calm steers (2.02, 0.25, 0.09, and 0.21 mg/mL, respectively). Furthermore, serum cumulative PUFA of Temperamental steers (4.47 mg/mL) was greater ($P = 0.003$) than Calm steers (3.69 mg/mL). Previous work in other fields of study have used PUFA as markers for stress responsiveness and inflammation in tissues. In agreement with those previous studies, markers of stress and inflammation were related with an increase in overall PUFA concentration in blood in the present study. These findings add to the current body of work regarding cattle temperament and associated alterations of metabolic components. It is not clear if elevated blood PUFA are directly impacting cattle immunity, performance, carcass traits, and/or meat quality among temperamental cattle. However, it is widely known that alteration of fatty acid composition in the final product has numerous organoleptic impacts. Future research is required to determine if circulating lipids in blood ultimately impact overall meat quality.

Key Words: blood metabolites, cattle, fatty acids, PUFA, temperament

1051 Effects of intrammamary LPS infusions on inflammation and reproductive parameters of dairy cows. C. C. Campos[*1], A. C. C. Fernandes[2], I. Hartling[3], M. Kaur[2], R. M. Dos Santos[4], R. L. A. Cerri[3], *[1]FAMEV-UFU, Uberlândia, Brazil, [2]Faculty of Land and Food Systems- University of British Columbia, Vancouver, BC, Canada, [3]Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada, [4]Universidade Federal de Uberlândia, Uberlândia, Brazil*

The objective was to determine the effects of LPS induced mastitis on systemic inflammatory response and early embryo development in lactating Holstein cows. Cows at 35 ± 7 DIM ($n = 20$) were submitted to a modified Double-Ovsynch program (10 d interval between protocols with CIDR and two PGF injections in the second protocol) and timed AI (d0). Cows were randomly assigned (block design) to two treatments: 1) LPS group- cows received an intrammamary infusions (d5 and d10) of 25 μg of LPS (strain 0111:B4) diluted in 10 mL of sterile saline at morning milkings; and 2) Control group- cows received infusion with saline. Blood samples were taken at different time intervals during the study to determine plasmatic concentrations of haptoglobin (Hp), tumoral necrosis factor α (TNF-α) and progesterone (P_4). Milk samples were collected every 2 d for somatic cell count (SCC) and body temperature was recorded using a rumen-reticular bolus logger and summarized for every hour during the period. On Day 15 after AI, uterine flushing for embryo recover and interferon-tau (IFN-τ) measurement, as well as endometrium biopsy were performed. Data were analyzed using the MIXED procedure of SAS. Hp was greater in LPS compared with Control group (0.80 ± 0.06 vs. 0.45 ± 0.07; $P < 0.01$), but TNF-α concentration was similar ($P = 0.72$) between treatments. Milk production from d0 to d15 was greater for Control cows (37.5 ± 1.5 vs. 33.5 ± 1.3 kg/d; $P < 0.01$), whereas SCC was higher in LPS treated cows for about 48 h after each infusion ($P < 0.01$). Likewise, reticular temperature of LPS cows was elevated for 12 h ($P < 0.01$) after each infusion. Progesterone did not differ among treatments at all time collections ($P = 0.72$). However, pregnant cows had greater concentrations of progesterone on d 6, 9, and 10 post-AI ($P < 0.01$). The recovery rate was 55% and the length of recovered embryos (3.6 ± 0.9 vs. 2.4 ± 0.7 cm; $P = 0.56$) and IFN-τ concentration in the luminal uterine flushing ($P = 0.44$) were similar between treatments. In summary, the intrammamary infusion of LPS was able to trigger a systemic inflammatory response during post-AI period, but unable affect conceptus recovery and length, and intraluminal uterine IFN-t concentration.

Key Words: embryo development, inflammation, LPS, mastitis

1052 Relationships of calf vigor at birth with calf size and circulating metabolites in fall-born beef calves. J. M. Larson*[1], B. L. Vander Ley[2], A. M. Meyer[1], [1]*Division of Animal Sciences, University of Missouri, Columbia, MO,* [2]*Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO*

To evaluate the relationship of neonatal calf vigor with calf size and circulating metabolites, 66 beef cows and heifers (average age = 4.4 ± 0.5 yr; average BCS = 5.2 ± 0.1; average calving date = September 11, 2015) were monitored during calving. Calf time to stand was determined from the time of birth until the time the calf successfully stood for 5 consecutive seconds (n = 30). Gestation length, birth weight, and body measurements (crown to rump length, shoulder to rump length, heart girth, abdominal girth, and cannon bone circumference) were measured. Jugular blood samples were obtained from 8 bull and 16 heifer calves from this subset at 0, 6, 12, 24, 48, and 72 h postnatally for plasma glucose and serum blood urea nitrogen (BUN), NEFA, albumin, total protein, and globulin analysis. Serum fructose was determined in 0 h samples only. Samples at 0 h were obtained before colostrum intake but after standing. Correlations were determined between time to stand (min) and all neonatal parameters. Birth weight had a moderate positive correlation (P = 0.01) with time to stand. Crown to rump length, shoulder to rump length, abdominal girth, and heart girth tended to have a weak positive correlation ($P \leq 0.11$) with time to stand. Gestation length and cannon bone circumference were not correlated ($P \geq 0.33$) with time to stand, and calf sex did not affect (P = 0.39) calf vigor. Time to stand had a moderate negative correlation with serum NEFA at 24 h (P = 0.04) and 72 h (P = 0.07). Circulating albumin at 0 h tended to have a moderate negative correlation (P = 0.06) with time to stand, and had a moderate or strong negative correlation ($P < 0.02$) with time to stand at the remaining sampling times. Plasma glucose from 12 through 72 h tended to have a weak or moderate positive correlation ($P \leq 0.15$) with time to stand. Time to stand had a moderate or strong positive correlation ($P \leq 0.05$) with BUN at 6 through 48 h. Total protein, globulin, and fructose concentrations were not correlated ($P \geq 0.25$) with time to stand. In conclusion, calf size may play a role in beef calf vigor at birth. Pre-suckling circulating metabolites appear to be poor predictors of vigor, but several neonatal metabolites after colostrum intake were related to vigor in this study.

Key Words: neonates, parturition, vigor

1053 Effect of pregnancy on steroid and eicosanoid metabolizing enzymes in bovine reproductive tissues. M. P. T. Coleson*[1], E. J. Northrop[2], J. J. J. Rich[2], G. A. Perry[2], C. G. Hart[1], K. J. McCarty[1], C. O. Lemley[1], [1]*Mississippi State University, Mississippi State, MS,* [2]*Department of Animal Science, South Dakota State University, Brookings, SD*

The objective was to determine the effects of pregnancy status on steroid and eicosanoid metabolizing enzymes in Angus cross cattle (between 3 and 13 yr) 16 d post-insemination within corpora lutea (**CL**) or endometrial (caruncle; **CAR** and inter-caruncle; **IC**) tissues. Cattle were fixed-time artificially inseminated. Cattle were further classified as either exhibiting or not exhibiting estrus based on estrus activity (confirmed with peripheral concentrations of estradiol). Sixteen d after AI cattle were euthanized and reproductive tracts collected from 18 non-pregnant and 10 pregnant cows (pregnancy determined by presence of an embryo). Activity of cytochrome P450 1A (**CYP1A**) and UDP-glucuronosyltransferase (**UGT**) enzymes were determined using specific luminogenic substrates. Activities were expressed relative to mg of protein or g of tissue. In addition, total activity of the CL was calculated by multiplying activity per g of tissue by CL weight. Data were analyzed using MIXED procedure of SAS and the model statement included pregnancy status, display of estrus, and the respective interaction. In the CL, activity of CYP1A relative to mg of protein, g of tissue, and CL total was not different ($P > 0.19$) between pregnant and non-pregnant cattle, as well as cattle that exhibited or failed to exhibit estrus. In CAR and IC, activity of CYP1A relative to mg of protein and g of tissue was not different ($P > 0.40$) between pregnant and non-pregnant cattle, as well as cattle that exhibited or failed to exhibit estrus. In the CL, activity of UGT relative to mg of protein was not different ($P > 0.14$) between pregnant and non-pregnant cattle, as well as cattle that exhibited or failed to exhibit estrus. However, in CL the activity of UGT relative to g of tissue and CL total decreased ($P < 0.05$) in pregnant vs. non-pregnant cattle which exhibited estrus, while it was not different in pregnant vs. non-pregnant cattle that did not exhibit estrus. In CAR and IC the activity of UGT relative to mg of protein and g of tissue was not different ($P > 0.15$) between pregnant and non-pregnant cattle, as well as cattle that exhibited or failed to exhibit estrus. In conclusion, activity of UGT was decreased in the CL of pregnant vs. non-pregnant cattle that exhibited estrus. This alteration in CL UGT activity could affect steroid and eicosanoid metabolism during early pregnancy.

Key Words: corpus luteum, cytochrome P450, pregnancy

1054 Effect of exogenous β-hydroxybutyrate in the lateral ventricle on circulating serum metabolites and luteinizing hormone in castrated lambs.

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Metabolic dysfunctions are known to have negative impacts on reproduction. It has been well established that during periods of fasting or nutrient restriction, reproduction is inhibited due to the suppression of pulsatile luteinizing hormone (LH) secretion. The manifestation of metabolic dysfunction through elevated β-hydroxybutyrate (BHB) concentrations could be an indication of poor adaptation to negative energy balance (NEB) and modulate reproductive incompetence in livestock. Therefore, the objective of this study was to evaluate the effect of a central injection of exogenous BHB into the lateral ventricle on circulating metabolic markers and LH secretion in lambs. Ten wether lambs were individually housed and fed once a day at a rate of 1.1 kg/d of a 13.5% CP and 72.5% TDN complete feed ration. Before experimental treatments, lambs were fitted with lateral ventricle intracerebroventricular brain cannulas. Lambs were centrally injected with 1 mL into the lateral ventricle with one of two treatments: (1) β-hydroxybutyric acid sodium salt solution (BHB; 12,800 μmol/L) or (2) saline solution (CON). Serum blood samples were collected every 10 min for 60 min before treatment injection and every 10 min for 120 min after infusions. Serum glucose concentrations increased ($P < 0.01$) with BHB injection, indicating stimulation of gluconeogenesis. Infusion of BHB also increased serum non-esterified fatty acid levels ($P < 0.01$). In addition, serum BHB concentrations also increased ($P < 0.01$) in lambs when infusion of BHB in the lateral ventricle occurred. Injection of BHB in the lateral ventricle tended ($P = 0.08$) to inhibit overall LH secretion (mean LH). Number of LH peaks during the 2 h sampling period after injection of treatments did not differ ($P = 0.18$) between lambs injected with BHB or CON. However, lambs injected with BHB had significantly decreased ($P < 0.01$) LH amplitudes. The results of this study suggest that elevated β-hydroxybutyrate in the brain mimics a negative energy signal leading to an increase in the mobilization of glucose and non-esterified fatty acids, while suppressing luteinizing hormone.

Key Words: β-hydroxybutyrate, energy sensing, metabolism, reproduction

1055 WS Comparisons of two short duration estrous synchronization protocols on pregnancy rates to fixed-time AI.

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The objective of the current experiment was to compare pregnancy rate and estrus response between a 5-d or 6-d CO-Synch + CIDR synchronization protocol. Multiparous cows ($n = 238$) were assigned to either a 5-d CO-Synch + CIDR (5-Day) or a PG 6-d CIDR (6-Day) groups based on body weight and body condition score (BCS). Cows assigned to the 5-d protocol were given GnRH (100 μg i.m., Factrel) at the time of insertion of a controlled internal drug releasing device (CIDR; Eazi-Breed CIDR). Five d later CIDR was removed and PGF2α (25 mg i.m., Lutalyse) was given with an additional injection of PGF2α 8 h after CIDR removal. Cows assigned to the 6-d protocol were given an injection of PGF2α and three d later a CIDR was inserted and an injection of GnRH was given. Six d later CIDR were removed and PGF2α was given. Estrus detection aids were applied at CIDR removal. Cows were inseminated by fixed-time AI (FTAI) with conventional semen 72 h after CIDR removal, and GnRH was administered at the time of AI. At insemination, estrus status was categorized as positive (YES), unknown (NR) or negative (NO). Cows were divided into three groups and bulls were introduced 14 d post-insemination at a 1:50 ratio. Bulls were removed 60 d after FTAI and pregnancy was determined by transrectal ultrasound. Pregnancy diagnosis was confirmed by palpation 60 d after the bulls were removed. The AI and final pregnancy rates averaged 62.6% and 95.0%, respectively, and were similar ($P < 0.7$) between 5-Day and 6-Day protocol. There was no difference ($P = 0.11$) in the percentage of cows expressing estrus between the treatments (42.4% and 54.9% for 5-Day and 6-Day, respectively). Expression of estrus before FTAI increased ($P < 0.05$) AI pregnancy rates by 21%; however, it did not increase ($P = 0.32$) final pregnancy rates. There was no interaction ($P = 0.11$) among synchronization protocols and expression of estrus on AI pregnancy rates. In conclusion, expression of estrus increased pregnancy rates; however, there was no difference in pregnancy rates between synchronization protocols.

Key Words: beef cows, estrous synchronization, fixed-time AI

1056 WS Effect of prostaglandin administration after ram exposure on ewe reproductive efficiency.
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A 2-yr experiment was conducted to determine the effect of a single injection of prostaglandin after ram turnout on ewe estrous synchronization. Rambouillet ewes (n = 100; yr 1 = 52; yr 2 = 48) at New Mexico State University West Sheep Unit were stratified by age and BW and assigned to 1 of 3 treatments: untreated (**CON**; n = 33); 12-d CIDR insert (**CIDR**; n = 33); or 1 injection of prostaglandin at d 2.5 (**1PG**; n = 34) after rams were placed with ewes. Ewes were exposed to rams at CIDR insert removal (d 0) for a 35-d breeding season. Ewes were observed twice daily to determine estrus. A greater ($P \leq$ 0.01) number of CIDR ewes were bred in the first 3 d of the breeding season (82%), compared with 1PG (35%) or control (21%) ewes. Moreover, there was an increased ($P \leq 0.01$) number of CIDR ewes bred in the first 4 d (94%) compared to 1PG (50%) ewes, both of which had an increased ($P \leq 0.01$) number of ewes bred compared to control ewes (21%). Both CIDR (94%) and 1PG (73.5) ewes had an increased number ($P \leq 0.01$) of ewes bred in the first 5 d compared to control (33%) ewes. As expected, CIDR-treated ewes had a shorter time (2.2 d) to breeding, than 1PG treat ewes (4.9 d) and control ewes took longer to breed than both CIDR and 1PG ewes (8.1 d) ($P \leq 0.01$). Lambing and weaning data have not yet been collected for yr 2. In yr 1 the number of lambs born per ewe and kg of lamb weaned per ewe was not different ($P \geq$ 0.33) between treatments. Based on these data, utilizing a single injection of PG 2.5 d after ram turnout resulted in similar pregnancy rates at d 5 of the breeding season when compared with CIDR-treated ewes suggesting in a confinement setting the 1PG synchronization protocol could potentially be utilized as a less expensive method of synchronization. Additional information will be collected to determine the effects of synchronization protocol on post-lambing data to determine efficacy of the treatments. Moreover, more research is needed to determine the efficacy of the proposed synchronization protocol in a range production environment.

Key Words: reproduction, sheep, synchronize

1057 The association between Anti-Mullerian Hormone concentrations, antral follicle count and fertility measures in dairy cows.
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The objectives were to (1) characterize variations in plasma concentrations of Anti-Müllerian Hormone (AMH) within a population of dairy cows, and (2) determine associations between AMH categories and AFC, first service conception rate, number of services and days open. Lactating Holstein cows (35 primiparous, 65 multiparous) were subjected to blood sampling (for plasma AMH; AnshLite Bovine AMH CLIA, Ansh Labs, Webster, TX) and transrectal ultrasonography (for AFC; 7.5 MHz linear array transducer; Aloka Co Ltd., Tokyo, Japan) at 75 ± 1 d postpartum (approximately 48 h after the second GnRH of an Ovsynch protocol). Cows were ranked in a descending order based on AMH concentrations and those in the top and bottom thirds were categorized into HIGH- (n = 33) and LOW-AMH (n = 33), respectively. The continuous variables (plasma AMH concentrations, AFC, number of services and days open) were analyzed using MIXED procedure of SAS. The association between AMH and AFC was tested using REG procedure, and the effect of categories of AMH and parity groups on first service conception rate was analyzed using GLIMMIX procedure. Plasma AMH concentrations (pg/mL) ranged from 38.2 to 774.1 (CV 63.4%) with an overall mean (± SEM) of 224.8 ± 14.3. Plasma AMH was greater in the HIGH-AMH than in the LOW-AMH category cows (386.2 ± 14.9 vs. 95.8 ± 15.2 pg/mL; $P < 0.01$). Mean AFC was also significantly greater in HIGH-AMH than those in LOW-AMH category cows (28.1 ± 1.4 vs. 16.7 ± 1.4; $P < 0.01$). Plasma AMH concentrations were linearly associated with AFC (R^2 = 37.5; $P < 0.01$). First service conception rate did not differ between AMH categories (HIGH-AMH vs. LOW-AMH: 35.3 vs. 36.4; $P > 0.05$). However, cows in the HIGH-AMH category tended to have fewer number of services (2.1 ± 0.2 vs. 2.7 ± 0.2; $P = 0.08$) and days open (133.6 ± 11.5 vs. 162.3 ± 11.7 d; $P = 0.09$) than those in LOW-AMH category. Parity did not influence plasma concentrations of AMH, AFC, first service conception rate, number of services or days open ($P >$ 0.05). In summary, plasma AMH concentrations were highly variable and associated with AFC; however, AMH categories did not influence first service conception rates although cows in the HIGH-AMH category had a tendency for fewer days

open and reduced number of services.

Key Words: anti-Müllerian hormone, antral follicle count, fertility

1058 Natural patterns of early postpartum luteal activity and their association with insemination outcomes in dairy cows.

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The objective of this study was to investigate associations between different patterns of luteal activity early postpartum, up to first service, and insemination (AI) outcomes. Milk progesterone (P4) concentrations measured through in-line milk analysis (Herd Navigator, DeLaval Inc) from 785 Holstein cows were assessed from two dairy herds. Progesterone > 5ng/mL was indicative of luteal activity (LA). Milk P4 was determined at set intervals starting 20d postpartum and cows that initiated LA at ≤ 35d and > 35d were considered to have ovulated early (Early-Ov) and late (Late-Ov), respectively. Luteal activity, until first service, lasting 7d to 19d was defined as normal and LA lasting < 7d or > 19d as abnormal. Outcomes of first and second AI were: pregnant (LA > 45d; 1stAI n = 237; 2ndAI n = 133), open (LA ≤ 19d; 1stAI n = 428; 2ndAI n = 337), or pregnancy loss (LA > 20d but ≤ 45d; 1stAI n = 120; 2ndAI n = 62). From calving to 1stAI, luteal phases were classified as at least one normal LA (1NormLA), at least two normal LA (2NormLA), at least one abnormal LA (1AbnLA) and at least two LA whether normal or abnormal (2TotLA). Each of the four categories of luteal phases were analyzed as binomial variables against AI outcomes, Early-Ov or Late-Ov, parity and interactions, using mixed-effects logistic regression (GLMER of R-3.2.3) with herd as random factor. Cows in 1NormLA had increased odds of being pregnant at 1stAI (odds ratio [OR]: 3.21, P = 0.001) and cows in 2NormLA had increased odds of being pregnant at first and 2ndAI (OR: 1.63, P < 0.03). AbnLA1 decreased the probability of pregnancy (OR: 0.66, P = 0.01) and tended to be associated with pregnancy loss at 2ndAI (66%, P = 0.08). Cows having 2TotLA had increased probability of being pregnant at first and 2ndAI (OR: 2.41 and 1.80, respectively, P < 0.01). Cows that were open at 1stAI had twice the odds to suffer pregnancy loss at 2ndAI than cows that suffered pregnancy loss at 1stAI (OR: 1.99, P < 0.03). Primiparous cows were more likely to become pregnant at 1stAI than multiparous cows (OR: 1.56, P < 0.01), while multiparous cows had higher odds of delayed ovulation (32.58%, P = 0.04). Early-Ov cows had higher odds of having 2TotLA (98%, P < 0.001) and lower odds of suffering pregnancy losses at 2ndAI (OR: 0.70, P = 0.05). In summary, an early onset, and an increased frequency of normal luteal activity preceding first AI postpartum benefits insemination outcomes.

Key Words: estrous cycle, fertility, milk progesterone profiles

1059 Circulating LH concentrations after intravaginal instillation of GnRH in lactating dairy cows.

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Our objective was to evaluate circulating LH concentrations after intravaginal (**IVG**) instillation of GnRH in dairy cows. Lactating primiparous (n = 6) and multiparous (n = 27) Holstein cows received two luteolytic doses of PGF2α 12 h apart 7 d after the Ovsynch protocol (GnRH-7d-PGF2α-56h-GnRH). Forty 8 h after the first PGF2α treatment cows were stratified by parity and randomly allocated to five different treatments: 2 mL of saline solution IVG (**SAL-IVG**, n = 6), 100 µg of GnRH i.m. (**G100-im**, n = 5), 100 (**G100-IVG**, n = 7), 500 (**G500-IVG**, n = 8), or 1000 µg of GnRH IVG (**G1000-IVG**, n = 7). For all GnRH treatments Gonadorelin diacetate tetrahydrate (Cystorelin) was used. Blood was collected using indwelling jugular catheters at −1 h, 0 h, every 15 min up to 4 h, and every 30 min from 4 to 6 h after treatment. Data for progesterone, estradiol, and LH concentrations were analyzed by ANOVA with (LH only) or without repeated measures using PROC MIXED of SAS. Concentrations of progesterone and estradiol did not differ (P > 0.10) among groups at time 0. Concentrations of LH were affected by treatment (P < 0.001), time (P < 0.001) and treatment by time interaction (P < 0.001). Cows in G100-im had greater (P < 0.05) mean LH than the IVG treatments from 15 to 195 min after treatment whereas the G1000-IVG group had greater (P < 0.05) mean LH than the SAL-IVG and the other IVG GnRH groups from 45 to 240 min (except at 60, 75, and 90 min) after treatment. Mean LH for SAL-IVG, G100-IVG, and G500-IVG did not differ (P > 0.05) at any time point. The greatest (P < 0.001) area under the curve (**AUC**) was observed for G100-im (1149 ± 51 ng) followed by G1000-IVG (546 ± 108 ng) which had greater AUC than the other IVG treatment groups (242 ± 40, 271 ± 34 and 247 ± 24 ng for SAL-IVG, G100-IVG, and G500-IVG, respectively). Mean LH peak was greater (P < 0.001) for G100-im (6.9 ± 0.4 ng/mL) than G1000-IVG (2.8 ± 0.6 ng/mL) whereas the SAL-IVG, G100-IVG, and G500-IVG groups did not have a discernible LH surge after treatment (maximum LH: 1.2 ± 0.2, 1.3 ± 0.2 and 1.2 ± 0.1 ng/mL for SAL-IVG, G100-IVG and G500-IVG respectively). We conclude that IVG instillation of 1000 µg of GnRH induced more LH release than IVG instillation of saline solution and a 100 or 500 µg dose of GnRH. Also, the amount of LH released after IVG treatment with 1000 µg of GnRH was less than that released after i.m. treatment with 100 µg of GnRH.

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Key Words: intravaginal, GnRH, LH

1060 Effect of dose and timing of prostaglandin $F_{2\alpha}$ treatments during a Resynch protocol on luteal regression and fertility to timed artificial insemination in lactating Holstein cows.

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Our objective was to evaluate the effect of a second $PGF_{2\alpha}$ treatment (25 mg dinoprost) or a double dose of $PGF_{2\alpha}$ (50 mg dinoprost) during a Resynch protocol on luteal regression and pregnancies per artificial insemination (P/AI) in dairy cows. Lactating Holstein cows (n = 1438) were randomly assigned at a nonpregnancy diagnosis to receive: 1) Ovsynch (Control: GnRH; 7 d, $PGF_{2\alpha}$; 56 h, GnRH); 2) Ovsynch with a second $PGF_{2\alpha}$ treatment (GPPG: GnRH; 7 d $PGF_{2\alpha}$; 24 h, $PGF_{2\alpha}$; 32 h, GnRH); or 3) Ovsynch with a double dose of $PGF_{2\alpha}$ (GDPG: GnRH; 7 d, 2x$PGF_{2\alpha}$; 56 h, GnRH). All cows received TAI ~16 h after the second GnRH treatment (G2). Pregnancy diagnosis was performed by transrectal palpation 38 ± 3 d after TAI, and pregnancy status was reconfirmed 28 d later. Blood samples were collected at the first $PGF_{2\alpha}$ treatment and at G2 from a subset of cows (n = 546) and assayed for progesterone (P4). Data were analyzed by logistic regression using the GLIMMIX procedure of SAS. At 38 d after TAI, GPPG cows tended to have more (P = 0.12) P/AI than Control cows [37% (181/495) vs. 33% (154/463)], whereas P/AI for GDPG cows [34% (164/480)] did not differ (P = 0.34) from Control cows. Pregnancy loss from 38 to 66 d did not differ (P = 0.46) among treatments and was 8% (38/475). The percentage of cows with complete luteal regression (P4 $\leq$ 0.3 ng/mL at G2) tended to differ (P = 0.06) among treatments and was greater for GPPG cows than for GDPG and Control cows (94% vs. 88% vs. 88%, respectively). Overall, cows with P4 < 1 ng/mL at the first $PGF_{2\alpha}$ treatment had fewer (P < 0.01) P/AI than cows with P4 $\geq$ 1 ng/mL [28% (40/145) vs. 41% (148/365)], whereas cows with P4 > 0.3 ng/mL at G2 had fewer (P < 0.01) P/AI than cows with P4 $\leq$ 0.3 ng/mL [14% (7/51) vs. 39% (167/425)]. We conclude that addition of a second $PGF_{2\alpha}$ treatment during a Resynch protocol tended to increase P/AI to TAI by increasing the percentage of cows with complete luteal regression at G2, whereas doubling the dose of $PGF_{2\alpha}$ did not. *Supported by USDA NIFA Hatch project 1006519*

Key Words: dairy cow, resynchronization, timed AI

1061 Fertility of lactating Holstein cows after synchronization of ovulation and timed artificial insemination versus artificial insemination after detection of estrus at a similar DIM range.

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Our objective was to compare pregnancies per artificial insemination (P/AI) at first service after synchronization of ovulation and timed artificial insemination (TAI) with artificial insemination (AI) after detection of estrus at a similar DIM range. Lactating Holstein cows (n = 408) were randomly assigned to receive their first TAI after a Double-Ovsynch protocol [TAI; Pre-Ovsynch (GnRH; 7 d, $PGF_{2\alpha}$; 3 d, GnRH) followed 7 d later by Breeding-Ovsynch (G1; 7 d $PGF_{2\alpha}$; 24 h, $PGF_{2\alpha}$; 32 h, GnRH; 16 h, TAI)] or to receive first AI after estrus induced using $PGF_{2\alpha}$(Estrus; $PGF_{2\alpha}$; 14 d, $PGF_{2\alpha}$; 24 h, $PGF_{2\alpha}$). Cows were inseminated using frozen-thawed semen from 4 AI sires with proven high fertility (sire conception rate > 0). Overall, 92% (374/408) of cows received their first insemination from 74 to 81 DIM. Estrus cows inseminated > 7 d after the last $PGF_{2\alpha}$ treatment (n = 34) were excluded from the analysis of P/AI but were included in the calculation of insemination rate. Pregnancy status was determined 33 ± 3 d after AI, and pregnancy status was reconfirmed 63 ± 3 d after AI. Data were analyzed by ANOVA and logistic regression using MIXED and GLIMMIX procedures of SAS. DIM at AI did not differ (P = 0.37) between treatments (76.9 ± 0.2 vs. 76.7 ± 0.3 for TAI vs. Estrus cows, respectively). More (P < 0.01) TAI cows received AI within 7 d after the VWP than Estrus cows (100% vs. 83%). At 33 d after AI, primiparous cows had more (P < 0.01) P/AI than multiparous cows [58% (81/139) vs. 37% (87/235)], and TAI cows had 50% more (P < 0.01) P/AI than Estrus cows [51% (109/212) vs. 34% (59/162)]. No parity by treatment interaction was detected (P = 0.20). At 63 d after AI, TAI cows had 39% more (P = 0.02) P/AI than Estrus cows [46% (69/149) vs. 33% (38/116)], and pregnancy loss from 33 to 61 d after AI did not differ (P = 0.16) between treatments [14% (11/81) vs. 5% (2/41) for TAI vs. Estrus cows]. In conclusion, synchronization of ovulation and TAI for first service increased the percentage of cows inseminated within 7 d after the VWP, and TAI cows had greater fertility at first service than Estrus cows at a similar DIM range. *Supported by USDA NIFA Hatch project 1006519 and CEVA Sante Animale*

Key Words: estrus, fertility, timed AI

1062 Increasing estrus expression in lactating dairy cows. J. A. Sauls*, B. E. Voelz, S. L. Hill, J. S. Stevenson, *Kansas State University, Manhattan, KS*

Using an activity monitoring system (AMS) equipped with an accelerometer, 2 experiments were conducted to test the hypotheses that: (1) altering progesterone before inducing luteolysis or (2) exposing cows to estradiol cypionate (ECP) or testosterone propionate (TP) after luteolysis would increase occurrence and intensity of estrus. In experiment 1, cows (n = 154) were fitted with an AMS collar and a pressure-sensitive device (HW) and assigned to 3 treatments: 1) CL only; 2) no CL + progesterone insert (CIDR); or 3) CL + 2 CIDR to achieve different concentrations of progesterone. Progesterone 24 h post-treatment was greatest ($P < 0.01$) in CL + 2 CIDR, followed by CL, and no CL + CIDR cows. Estrus occurred 11 to 12 h earlier ($P < 0.01$) in no CL + CIDR compared with CL-bearing cows. Estrus intensity was greater ($P \leq 0.05$) after CL + 2 CIDR than CL only cows. The AMS and HW determined 68 and 62% of the qualifying cows to be in estrus (estrus was defined: follicle $\geq$ 10 mm at $PGF_{2\alpha}$ and progesterone $\leq$ 0.5 ng/mL 72 h later), respectively. In experiment 2, cows (n = 203) were equipped with an AMS and a friction-activated patch (Estrotect patch; P) and assigned to receive 1 mg ECP, 2 mg TP, or control 24 h after $PGF_{2\alpha}$. Estradiol 24 h post treatment was greater ($P <$ 0.01) in ECP compared with controls. Estrus expression detected by P in all cows tended ($P = 0.10$) to be greater for ECP compared with controls. More ($P < 0.05$) qualifying cows were detected in estrus after ECP compared with controls. Compared with controls and in response to ECP, estrus occurred 17 to 20 h earlier ($P < 0.01$) and was of greater ($P < 0.05$) intensity. The AMS and P determined 71% and 74% to be in estrus, respectively. Of cows exposed to the AMS, HW, or P, 62 to 74% were detected in estrus and more than 94% ovulated. In contrast, of the residual cows not detected in estrus, 60 to 76% ovulated in the absence of detected estrus. Only ECP was successful in inducing more estrus expression, but proportions never exceeded 80%. Given the large proportion of cows ovulating in the absence of estrus, further research is warranted to determine if conception is achievable by inseminating cows not detected in estrus by 72–80 h post-PGF_{2a}.

Key Words: estrus, estradiol, progesterone

1063 The characterization of estradiol concentration before insemination and its effect on fertility in dairy cattle. M. Gobikrushanth*[1], P. A. Dutra[1], C. A. Felton[2], T. C. Bruinjé[1], M. G. Colazo[2], S. Butler[3], D. J. Ambrose[1,2], *[1]Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Livestock Research Branch, Alberta Agriculture and Forestry, Edmonton, AB, Canada, [3]Animal & Grassland Research and Innovation Centre, Teagasc Moorepark, Fermoy, Co. Cork, Ireland*

The objectives of this study were to: (1) characterize variations in plasma concentrations of estradiol (E2) within a population of dairy cows, and (2) determine associations between E2 categories, LH, ovulatory response, first service conception rate, number of services and days open. Lactating Holstein cows (35 primiparous, 65 multiparous) received one injection of $PGF_2\alpha$ (cloprostenol, 500 µg, d 0) followed by GnRH (gonadorelin, 100 µg, d 3; Presynch) and were subjected to an Ovsynch protocol starting on d 10, with timed-AI (TAI) occurring at ~75 d postpartum. Blood samples were collected immediately before (0h) and 2h after the second GnRH of Ovsynch to determine plasma E2 and LH concentrations, respectively. Cows were ranked based on plasma E2 concentrations, from highest to lowest, and those in the top (n = 33) and bottom (n = 33) thirds were classified into HIGH- and LOW-E2 categories. The continuous variables were analyzed using MIXED procedure of SAS and the binomial data were modeled against E2 categories and parity and analyzed using the GLIMMIX procedure of SAS. Plasma E2 concentrations (pg/mL) ranged from 0.1 to 9.2 (CV 78.7%) with an overall mean (± SEM) of 2.0 ± 0.1. The plasma E2 was greater for cows in HIGH-E2 category than those in LOW-E2 category (3.7 ± 0.2 vs. 0.5 ± 0.2 pg/mL; $P < 0.01$). Similarly, cows in the HIGH-E2 category had greater concentrations of plasma LH than cows in the LOW-E2 category (13.5 ± 1.0 vs. 5.9 ± .1.0 ng/mL; $P < 0.01$). The categories of E2 did not influence ovulatory response to the second GnRH of the Ovsynch ($P <$ 0.05). The first service conception rate tended to be greater for cows in HIGH-E2 category than those in LOW-E2 category (44.1 vs. 24.2; $P = 0.10$). Further, cows in the HIGH-E2 category had a tendency for fewer number of services (2.2 ± 0.2 vs. 2.7 ± 0.2; $P = 0.10$) and lesser days open (138.6 ± 11.6 vs. 171.5 ± 11.7 d; $P = 0.06$) than cows in LOW-E2 category. Parity did not influence plasma concentrations of E2 and LH, ovulatory response to the second GnRH of Ovsynch, number of services or days open ($P > 0.05$). However, primiparous cows tended to have greater first service conception rate than multiparous cows (50.0 vs. 25.6%; $P = 0.06$). In summary, plasma E2 concentrations were highly variable in a tested population of dairy cows and cows in the HIGH-E2 category had a tendency for greater first service conception rate and

fewer number of services and lesser days open.

Key Words: estradiol concentration, fertility, variability

1064 Resynchronization of ovulation strategies including or not including GnRH treatment before non-pregnancy diagnosis. R. Wijma*, M. L. Stangaferro, M. Masello, G. E. Granados, J. O. Giordano, *Department of Animal Science, Cornell University, Ithaca, NY*

Our objectives were to evaluate ovarian dynamics and reproductive performance of cows managed with two different resynchronization protocols for second and greater AI services. After each AI, cows were randomly assigned to receive (**G25** group; $n = 649$) or to not receive GnRH (**NoG25**; $n = 656$) 25 ± 3 d after AI and 7 before non-pregnancy diagnosis (**NPD**) by transrectal ultrasonography. Non-pregnant cows in G25 ($n = 353$) and NoG25 ($n = 353$) received the same protocols as follows: cows with a corpus luteum (**CL**) $\geq$ 15 mm received two PGF treatments 24 h apart, GnRH 32 h later, and timed AI (**TAI**) 16 h after GnRH whereas, cows without a CL $\geq$ 15 mm at NPD received the CIDR-Ovsynch protocol (GnRH+CIDR-7d-CIDR-removal+PGF-24h-PGF-32h-GnRH-16h-TAI). Cows in both groups were inseminated to estrus (**AIE**) any time after AI. Circulating concentrations of progesterone (**P4**) were determined and ovarian ultrasonography was performed thrice weekly from 18 ± 3 d after AI until NPD in 44 and 46 cows for G25 and NoG25, respectively. Binomial outcomes were analyzed by logistic regression and continues outcomes using ANOVA. Overall, more cows ovulated ($P < 0.01$) spontaneously or in response to GnRH for G25 (69.9%) than NoG25 (36.4%) from 18 ± 3 d after AI to NPD, the proportion of cows with a CL tended ($P = 0.06$) to be greater for G25 (89.1%) than NoG25 (72.7%), and the proportion of cows with P4 > 1 ng/mL at NPD was similar ($P = 0.14$) for G25 (67.4%) and NoG25 (61.4%). A similar ($P = 0.74$) proportion of cows had an active follicle (**AF**, > 10 mm in the growing or static phase) at NPD (G25 = 91.3% and NoG25 = 95.5%) but size of the AF was greater ($P = 0.02$) for NoG25 (16.5 ± 0.6 mm) than G25 (15.0 ± 0.4 mm). For all cows enrolled, more ($P = 0.04$) non-pregnant cows after AI received AIE in NoG25 (55.8%, 169/353) than G25 (47.9%, 197/353) but more cows had a CL ($P = 0.01$) at NPD for G25 than NoG25 [83.7% (154/184) vs. 72.4% (113/155)]. Pregnancies per AI were similar for G25 and NoG25 for cows AIE [36.5% (51/167) vs. 38.7% (74/191); $P = 0.77$], cows with a CL at NPD [40.0% (58/145) vs. 33.0% (35/106; $P = 0.26$], cows without a CL at NPD [37.9% (11/29) vs. 39.0% (16/41); $P = 0.0.92$] or for all services combined [38.1% (130/341) vs. 36.9% (125/338); $P = 0.69$]. We conclude that despite differences in ovarian function, fertility after TAI and overall P/AI was similar for cows that received or not received GnRH 25 ± 3 d after AI.

Key Words: dairy cow, resynchronization, timed AI

1065 Effects of modification of proestrus length and duration of progesterone exposure on automated measurements of estrous expression in lactating Holstein cows. B. F. Silper*, T. A. Burnett, P. F. M. P. Souto, M. S. Baylao, A. P. O. Santos, R. L. A. Cerri, *Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada*

Objectives were to investigate if longer proestrus, via longer exposure to pre-ovulatory estradiol concentrations, would induce behavioral estrus of greater intensity, and to investigate if longer exposure to progesterone would offset possible effects of shorter proestrus on estrous behavior. Three treatments consisting of different intervals between GnRH-PGF (5 or 7 d) and PGF-ECP (1 or 3 d) on a Heatsynch program were applied to lactating Holstein cows ($n = 48$). All cows were fitted with three activity monitors (Heatime, SmartDairy, and AfiActII) and enrolled into pre-synchronization at 40 ± 3 DIM, followed by random allocation to treatments at 57 ± 3 DIM. Treatments were 5d3d (GnRH-5d-PGF-3d-ECP), 5d1d (GnRH-5d-PGF-1d-ECP), and 7d1d (GnRH-7d-PGF-1d-ECP). The AI was performed at a fixed time 48 h after ECP, except for those detected in high activity before timed-AI. Ovarian ultrasonography and blood samples were performed at injection days, day of AI, and 2, 5, and 7 d after timed-AI. Rates of estrous detection, ovulation (until 84 h after ECP) and presence of CL 7 d after timed-AI were not different among treatments ($P > 0.05$). Although the goal of 5d3d was to increase proestrus length, 6 of the 10 cows that showed high activity in this treatment did so before or at the day of ECP. Consequently, proestrus length was not different among treatments (3.0 ± 0.2, 3.2 ± 0.2, and 2.7 ± 0.2 d, for 5d1d, 5d3d, and 7d1d, respectively; $P > 0.05$). Pre-ovulatory follicle diameter was not significantly different among treatments and was 20.9 ± 0.7 mm (multiparous) and 19.2 ± 0.9 mm (primiparous). Odds of estrous expression were not influenced by parity, BCS, or progesterone concentration and follicle diameter at the PGF injection. Parity and BCS were the factors most frequently associated with measurements of estrus. Cows in 5d1d had estrus of greater duration (Heatime) than 7d1d (13.9 ± 0.9 vs. 11.1 ± 0.9 h; $P = 0.04$), whereas intensity of estrus (AfiActII) was greater for 5d3d cows (377.3 ± 43.0; $P = 0.04$) and tended to be greater for 7d1d (350.8 ± 41.3; $P = 0.09$), both in comparison with 5d1d (243.3 ± 45.8). Although estrous detection rate and proestrus length were not different among treatments, the use of a Heatsynch program with different GnRH-PGF or PGF-ECP intervals was capable of altering duration and intensity of estrus, likely due to changes in the endocrine profile during the estrous cycle.

Key Words: automated estrous detection, estradiol, proestrus

1066 Effect of GnRH removal at CIDR insertion in the 5 d CO-Synch + CIDR ovulation synchronization protocol on ovarian function in beef cows.

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The objective of this experiment was to evaluate the effects of GnRH removal at controlled internal drug release (CIDR) insertion in the 5-d CO-Synch + CIDR protocol (5dCO) on ovarian follicle growth and circulating steroid hormone concentrations. Non-pregnant, non-lactating beef cows (n = 15) were used in a 3 × 3 Latin square design and assigned to treatment by age and BCS to receive either: 1) standard 5dCO hormone administration including 100 μL of GnRH at CIDR insert and 2 concurrent 25-mg doses of $PGF_{2\alpha}$ (PG) delivered at CIDR removal (G2PG), 2) no GnRH at CIDR insert and 2 concurrent, 25-mg doses of PG at CIDR removal (NoG2PG), or 3) no GnRH at CIDR insert and a single, 25-mg dose of PG at CIDR removal (NoG1PG). All cows were monitored for estrous for 72 h after CIDR removal at which time 100 μL of GnRH was administered. Cows underwent transrectal ultrasonography to record ovarian structures and blood samples were taken for progesterone and estradiol analyses at CIDR insertion, CIDR removal, final GnRH administration, and d 5 and 10 post PG administration. An additional blood sample was collected on cows that displayed estrus between PG and GnRH. Data were analyzed using the MIXED and GLIMMIX procedures of SAS for the continuous and binary response variables, respectively. Dominant follicle diameter did not differ between treatments at CIDR removal or final GnRH ($P \geq 0.61$). Percentage of animals that regressed their corpus luteum (CL) in response to PG, estrus detection aid score, and CL volume 10 d following PG administration did not differ between treatments ($P \geq 0.18$). Post ovulation plasma progesterone did not differ between treatments ($P \geq 0.29$), and plasma estradiol was not different at CIDR removal or final GnRH administration ($P \geq 0.59$). However, peak plasma estradiol concentrations were greater ($P = 0.01$) in NoG2PG than NoG1PG (5.85 and 2.93 ± 0.55 pg/mL, respectively), with G2PG intermediate (3.31 ± 0.55 pg/mL). In conclusion, follicle and CL growth as well as subsequent progesterone concentrations were not negatively affected by removal of the initial GnRH in the 5-d CO-Synch + CIDR protocol; however fluctuations in estradiol concentrations merit implementation of a field trial to elucidate protocol impacts on fertility.

Keywords: 5-d CO-Synch, GnRH, Synchronization

1067 Effect of eCG and P4 level in timed AI programs in bos indicus and bos indicus x bos taurus heifers.

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This study evaluates the effects of P4 level and eCG treatment in timed-AI in Bos indicus (Nellore) and Bos indicus x Bos taurus (crossbred) heifers. Heifers used in the study (n = 1989) included Nellore (n = 992) and Crossbred (n = 997) that were 14–24 mo of age (BCS: 3.08 ± 0.01, BW: 329.09 ± 0.66 kg). Ovarian ultrasonography was performed twice (7 d apart) in all heifers at start of the study to identify heifers with a CL. Heifers with a CL were submitted to estrous synchronization and timed AI. Heifers without a CL in either ultrasonography were submitted to a puberty induction protocol (Rodrigues et al., 2014). Heifers with a detectable CL 12 d after puberty induction remained in the study. Timed-AI program was as follows: D0– Insertion of an intravaginal P4 device (CIDR 1.9 g; Zoetis, Sao Paulo, Brazil) and 2 mg of estradiol benzoate (Gonadiol; Zoetis); D7– 12.5 mg of dinoprost tromethamine (Lutalyse; Zoetis); D9– CIDR withdrawal and 0.5 mg of ECP (ECP; Zoetis). At D9 heifers were randomly assigned to receive either 0 (Control; 994) or 200 IU (eCG; 995) of eCG (Novormon; Zoetis); D11– timed AI was performed. On Days 9 and 11, a subgroup of heifers were evaluated by ultrasonography to record the largest follicular diameter. Continuous variables were analyzed using the PROC MIXED and binomial variables using the PROC GLIMMIX, both from SAS. Included in the models were effects of breed, group, eCG and serum P4 level (determined by ROC curve). Significance set when $P < 0.05$. Follicle diameter at D9 was greater for crossbred heifers (10.1 ± 0.01 mm) than Nellore heifers (9.5 ± 0.01) and in LowP4 (10.0 ± 0.01) compared to HighP4 (9.5 ± 0.01). Follicle diameter at D11 were not affected by tested variables. Ovulation rate was greater for eCG compared to Control (92.3% vs. 87.5%, respectively). Crossbred heifers had greater conception rate (63.7% vs. 56.3%) and greater pregnancy rate than Nellore (58.2% vs. 50.7%). eCG treatment (62.3%) tended to have greater conception than Control (55.3%). LowP4 heifers have greater conception and pregnancy rate than HighP4 (64.8, 59.6% and 55.3, 49.4%, respectively). Differences between Crossbred and Nellore heifers synchronized with the same timed AI program were observed, regardless of breed low P4 environments resulting in increased pregnancy rates. Acknowledgment: FAPESP process n° 2014/05270–9.

Keywords: bos indicus, bos taurus x bos indicus, timed-AI

1068 Gonadal and extra-gonadal sperm characteristics of rabbit bucks fed raw or fermented cottonseed cake– cased diets supplemented with ginger (Zingiber officinale Roscoe). A. A. Olajide*, *Ladoke Akintola University of Technology (Lautech), Ogbomoso, Nigeria*

The potential of cottonseed cake (CSC) as a veritable source of protein, energy and fiber for farm animals has been demonstrated. However, its use is limited to ruminant feeding due to the presence of gossypol, a polyphenolic compound of significant physiological implications. Fermentation is one of the biotechnological options for detoxifying a variety of feed ingredients. This study was conducted to investigate the effect of raw or fermented CSC– based diets (with or without ginger supplementation) on gonadal and extra-gonadal sperm characteristics of rabbit bucks. Thirty (30) cross-bred (New Zealand White X Chinchilla) rabbit bucks, 6 −7 wk old and averagely weighing 768.54 g, were randomly assigned to five dietary treatments (n = 6 per treatment) in a 2 × 2 factorial within a completely randomized design (CRD). The CSC replaced soyabean meal (SBM) at 0% (control) and 100% (Raw or Fermented) with or without ginger supplementation (30mg/kg feed). Animals were fed for 12 wk after which they were slaughtered; and their reproductive organs removed and processed for sperm evaluation. Raw CSC without ginger supplementation resulted in lower ($p < 0.05$) sperm count, Gonadal Sperm Reserve (GSR), Daily Sperm Production (DSP); and higher ($p < 0.05$) dead sperm than other treatments. The sperm count (69.33×10^6), motile sperm (79.97%), GSR (66.44×10^6) and extra-gonadal sperm reserve (109.98×10^6) were highest for bucks that were fed with fermented CSC with ginger supplementation. This study shows that total replacement of SBM with raw CSC reduced sperm quality in rabbit bucks. This adverse effect was corrected by a combination of fermentation and ginger supplementation.

Key Words: Rabbit bucks, Cottonseed cake, Sperm characteristics

1069 Supplementation with a Lactobacillus acidophilus fermentation product alters the metabolic response following a lipopolysaccharide challenge in weaned pigs. N. C. Burdick Sanchez*[1], J. A. Carroll[1], P. R. Broadway[1], B. E. Bass[2], J. W. Frank[2], *[1]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, [2]Diamond V, Cedar Rapids, IA*

This study was designed to determine if feeding a *Lactobacillus acidophilus* fermentation product to weaned pigs would alter the metabolic response following a lipopolysaccharide (LPS) challenge. Pigs (n = 30; 6.4 ± 0.1 kg BW) were housed individually with ad libitum access to feed and water. Pigs were weighed on arrival, assigned to 1 of 3 groups (n = 10/treatment), and fed for 18 d: 1) Control, fed a non-medicated starter diet; 2) Control + *Lactobacillus acidophilus* fermentation product at 1 kg/MT (SGX1; Diamond V SynGenX, Cedar Rapids, IA), and 3) Control +*Lactobacillus acidophilus* fermentation product at 2 kg/MT (SGX2). Pigs were anesthetized on d 7 and 14 for insertion of an i.p. temperature device and jugular catheter, respectively. On d 15, pigs were challenged i.v. with LPS (25 μg/kg BW LPS from *E. coli* O111:B4). Blood samples were collected at 0.5 h intervals from −2 to 8 h and at 24 h relative to LPS administration at 0 h and serum isolated for glucose, NEFA, and blood urea nitrogen (BUN) analysis. There was a treatment x time interaction ($P < 0.001$) for serum glucose; Control pigs had greater glucose than SGX1 and/or SGX2 pigs at 0.5, 1.5, 2.5, 4, 5, and 6.5h post-LPS ($P \leq 0.04$), while SGX2 had greater glucose than SGX1 at 3.5 h ($P = 0.02$). Baseline (−2 to 0h) NEFA was affected by treatment ($P < 0.001$) such that SGX1 pigs had the greatest (0.12 ± 0.01mmol/L) followed by Control (0.10 ± 0.01 mmol/L) and SGX2 pigs (0.08 ± 0.01 mmol/L). Thus, NEFA were analyzed as the change relative to baseline values. There were treatment ($P = 0.006$) and time ($P < 0.001$) effects for the change in NEFA; Control (0.23 ± 0.02 mmol/L) and SGX2 (0.27 ± 0.02 mmol/L) pigs had a greater change in NEFA than SGX1 pigs (0.15 ± 0.02 mmol/L). Baseline serum BUN was affected by treatment ($P < 0.001$); Control (12.62 ± 0.41 mg/dL) and SGX2 (13.26 ± 0.35 mg/dL) pigs had greater BUN than SGX1 pigs (10.86 ± 0.40 mg/dL); thus serum BUN were analyzed as the change relative to baseline values. There was a treatment x time interaction ($P = 0.004$) for the change in BUN; SGX1 pigs had greater BUN than Control and/or SGX2 at 0.5, 4, 5 to 6, 7, 7.5, and 24 h post-LPS challenge. These data demonstrate that feeding a *Lactobacillus acidophilus*fermentation product to weaned pigs may alter the redistribution of energy stores in response to an immune challenge which may expedite recovery.

Key Words: *Lactobacillus acidophilus* fermentation product, lipopolysaccharide, metabolism

1070 Non-targeted metabolomic evaluation of the uterine milieu during the transitional period of embryo elongation in the pig. J. R. Miles*[1], E. C. Wright-Johnson[1], T. D. Laughlin[2], C. D. Broeckling[3], L. A. Rempel[1], A. K. Pannier[2], *[1]USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, [2]Department of Biological Systems Engineering, University of Nebraska, Lincoln, NE, [3]Proteomics & Metabolomics Facility, Colorado State University, Fort Collins, CO*

Alterations in the signaling of critical molecular factors within the uterine milieu lead to deficiencies in embryo elongation. The objective of this study was to identify metabolites within the uterine environment that are present as porcine embryos transition between spherical, ovoid, and tubular embryos at Day 9, 10, and 11 of gestation, respectively. White crossbred

gilts (n = 9) were bred at standing estrus (designated d 0) and again 24 h later and randomly assigned to collection group. At Day 9, 10, or 11 of gestation (n = 3 per day), reproductive tracts were collected immediately following harvest and flushed with 40 mL of RPMI-1640 media. Embryo morphologies were assessed for each pregnancy to ensure gilts were assigned to the correct gestation day treatment group (i.e., Day 9 contained only spherical conceptuses, Day 10 contained only ovoid conceptuses, and Day 11 contained a mixture of ovoid and tubular conceptuses). Subsequent uterine flushings were submitted for non-targeted profiling by GC–MS and UPLC-MS techniques. Raw spectral data was processed using XCMS package in R and features were clustered using RAMclustR. Unsupervised multivariate principal component analysis (PCA) was performed in R using pcamethods package and univariate ANOVA was performed in R with a Benjamini-Hochburg false discovery rate (FDR) adjustment. Multivariate analysis of both the GC–MS and UPLC-MS spectral data demonstrated sample grouping that reflected the day of gestation. Maximum separation for the GC–MS data over time was observed with PC1 vs. PC2 accounting for 90% of the variance and PC2 identified several significant (P = 0.03) putative metabolites that changed over time. For the UPLC-MS data, separation over time was not as obvious but PC2 vs. PC6 did account for 28% of the variance and PC2 identified some significant (P = 0.04) metabolites that changed with time. After FDR adjustment of the GC–MS and UPLC-MS data, only C553, an unknown compound, was significantly (P = 0.02) greater in Day 11 uterine flushings compared to Day 9 and 10. However, several annotated compounds were trending toward differences, including aminomalonic acid which tended to be increased (P = 0.07) in Day 11 uterine flushings compared to Day 9 and 10. In conclusion, these data illustrate putative metabolites that change within the uterine milieu as porcine embryos transition between spherical, ovoid, and tubular embryos. USDA is an equal opportunity provider and employer.

Key Words: embryo elongation, metabolome, uterine milieu

1071 Effect of neuromedin u on pig immune regulation.

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Neuromedin U (NMU) is a conserved mammalian neuropeptide discovered in the 1980s, and found in two forms, NMU-25 and NMU-8. Wide distribution of NMU in animal organs suggests that NMU is involved in multiple physiological functions, including immue regulation. However, the role of NMU in pig immune regulation is still largely unknown. To study the effect of NMU on pig immune regulation, we cloned and detected the expression of NMU and its receptors in pig lymphatic organs and immune cells. We also investigated the effect of NMU on cytokine secretion after injection of (0, 5, 15, 45 nmol) NMU via intracerebral ventricle (i.c.v) into 16 pigs (n = 4 within each group), and the effect of (0.1 × 1000 nM) NMU on cytokine secretion in cultured dendritic cells and natural kill (NK) cells using ELISA and RIA methods. The results were as follows: 1. NMU and its receptors are expressed in lymphatic organs, cultured dendritic cells and NK cells. 2. Compared with the control group, NMU stimulated (P < 0.05) IL-1β, IL-6, IL-8, TNF-α and IL-10 secretion post-injection in a time- and dose-dependent manner. 3. NMU increased IL-8, IL-6 and IL-13 secretion and reduced IL-10 secretion (P < 0.05) in cultured dendritic cells. 4. NMU enhanced the killing activity of cultured NK cells, stimulated IFN-γ secretion and inhibited IL-10 secretion (P < 0.05) in NK cells in a time- and dose-dependent manner. Results from this study suggest that NMU has a role in pig immune regulation through its effect on cytokine secretion and increasing killing activity of NK cells.

Key Words: cytokine, immune regulation, NMU

1072 Evaluation of immune function of circulating leukocytes during the transition period in dairy cows.

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The aims of the study were to determine immune function circulating leukocytes using an ex vivowhole blood stimulation assay (WBA) with lipopolysaccharide (LPS) and assess gene expression profiles by RNA sequencing in the transition period of dairy cows. The WBA was performed on whole blood of 6 Holstein multiparous cows at −20, −3, 3, 7 d from parturition (DFP) using 0, 0.01 and 5 μg LPS/mL. The plasma collected after stimulation was used to analyze IL-1β and IL-6 concentration via ELISA. The data were analyzed as a factorial design with repeated measures, using PROC MIXED in SAS. At the same day of the WBA test, RNA was isolated from whole blood and sequenced on a Hiseq1000 (Illumina, USA). Differential gene expression analysis was conducted with the edgeR package, and a general linear model was applied considering −20 DFP as the baseline. A threshold of 1.5-fold change and P < 0.05 were used to define differentially expressed genes (DEG), which were subsequently analyzed through the Dynamic Impact Approach (DIA) using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The IL-1b and IL-6 released after LPS stimulation was higher at −3 DFP (P < 0.05) in comparison with −20 DFP. After calving, the response of IL-1β to stimulation of LPS decreased markedly, while the IL-6 response was unchanged up to 3 DFP (P < 0.01 vs. -20 DFP) and then declined at 7 DFP. The most-impacted and activated KEGG pathways highlighted by the DIA

analysis at −3 vs. −20 DFP were: PPAR signaling, adipocytokine signaling, hematopoietic cell lineage, ECM-receptor interaction and phagosome. After calving (3 and 7 DFP) the impact and activation of the above listed pathways was strongly increased, but there also was a strong inhibition of arachidonic acid metabolism (in particular enzymes regulating leukotrienes synthesis) as well as glycine, serine and threonine metabolism. Overall, the WBA and transcriptomic data confirmed changes in immune-competence of the circulating leukocytes around calving and, in particular, indicate mainly an increase of their activity and function. These data support the idea that the dairy cow's immune system is dysfunctional but not immunosuppressed around calving.

Key Words: Immune system, transition period dairy cows, transcriptomics, whole blood stimulation assay

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1073 Branched-chain amino acids (BCAA) in serum and skeletal muscle and mRNA expression of BCAA catabolizing enzymes in muscle of dairy cows around parturition.

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The BCAA (Leu, Ile, and Val), besides being substrates for protein synthesis or generation of energy, also act as signaling molecules and modulate overall AA and protein metabolism. The BCAA are mainly catabolized in skeletal muscle by transamination, followed by an irreversible oxidative decarboxylation by the action of branched-chain α-keto acid dehydrogenase complex (BCKDH). In view of the substantial mobilization of muscle protein during the transition period and the specific requirements of BCAA for milk protein synthesis and for supporting immune functions, our objective was to investigate the changes in the expression of BCAA catabolizing enzymes in conjunct with BCAA profiles during late gestation and the subsequent lactation in dairy cows. Biopsies from *M. semitendinosus* and blood were collected from 11 multiparous German Holstein cows on d −21, 1, 21, and 70 relative to calving. The BCAA profiles in muscle and serum were determined by LC-MS/MS profiling through targeted metabolomics using the Biocrates Absolute IDQ p180 Kit. The mRNA abundance of BCKDHA and BCKDHB was quantified by qPCR. Data were analyzed using the MIXED procedure of SAS. The concentrations of Leu, Ile, and Val in muscle increased from d −21 to d 1 ($P < 0.001$), remained unchanged until d 21 and then declined (except in case of Val) on d 70 ($P < 0.001$). In serum, the concentrations of Val and Ile changed over time ($P < 0.01$ and 0.06, respectively), whereas Leu remained stable. The mRNA abundance of BCKDHA decreased ($P = 0.03$) from d −21 to d 1, followed by a 1.9-fold increase ($P < 0.01$) on d 21 and then again declined thereafter ($P < 0.01$). The abundance of BCKDHB mRNA decreased 1.7-fold from d −21 to d 1 ($P < 0.01$), remained at this level on d 21, and then increased ($P < 0.01$) to nearly prepartum values on d 70. Negative correlations ($P < 0.01$) between BCKDHB mRNA abundance and muscle concentrations of Leu, Ile, and total BCAA ($r = −0.48$, −0.53, and −0.44, respectively) were observed across all time-points. Reduced abundance of BCKDHA and BCKDHB mRNA coincided with greater muscle concentrations of BCAA, suggesting an attenuation of BCAA oxidation in skeletal muscle shortly after parturition. This would favor sparing of BCAA for milk protein synthesis and other metabolic processes.

Key Words: branched chain amino acids, dairy cow, skeletal muscle

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1074 Incidence and risk factors related to anovulation in dairy cows.

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This study evaluated incidence and risk factors associated with anovulation in lactating dairy cows. Primiparous ($n = 357$) and multiparous ($n = 585$) Holsteins (43.6 ± 11.0 kg/d of milk and body condition score at 35 ± 3 days in milk [DIM] of 2.9 ± 0.3 [mean ± sd) had ovaries scanned at 35 ± 3 and 49 ± 3 DIM at PGF2α treatments of the Presynch to determine presence of corpus luteum and diameter of the largest follicle. Cows without corpus luteum at both examinations were considered anovular and classified in phenotypes according to follicle diameter: 4 to 8 mm; 9 to 14 mm; 15 to 24 mm; and ≥ 25 mm. The following information was collected until 49 ± 3 DIM. Dry period, incidence of retained placenta, metritis, ketosis, mastitis, lameness, respiratory and digestive problems. Cows detected in estrus until 5 d after the second PGF2α of the Presynch were inseminated, and the other cows were subjected to a fixed-time artificial insemination (FTAI) protocol. Categorical data were analyzed by logistic regression using the GLIMMIX procedure of SAS, and continuous data were analyzed using of the MIXED procedure ($P < 0.05$). The incidence of anovulation was 28.5% (268/942), and the distribution of phenotypes was 4.1% (11/268), 27.6% (74/268), 59.7% (160/268), and 8.6% (23/268) for 4 to 8 mm, 9 to 14 mm, 15 to 24 mm, and ≥ 25 mm, respectively. There was

a positive linear effect of dry period, and a negative linear effect of BCS on the incidence of anovulation. Milk production level was not associated with anovulation. Less healthy cows were anovular as compared to cows with history of one or multiple diseases (17.9%[c] [61/341], 29.8%[b] [94/315], and 39.5%[a] [113/286], respectively). All evaluated diseases were associated with increased incidence of anovulation when analyzed separately. A lower proportion of anovular cows was inseminated by estrus detection (27.1% [49/181] vs. 63.5% [344/542]) and, regardless of AI type, anovular cows were inseminated later (73.0 vs. 62.0 DIM) than cyclic cows. There was no difference in pregnancy per AI (P/AI) on d 60 of cows inseminated after estrus detection between anovular and cyclic cows (16.7% [8/48] vs. 18.1% [62/342], respectively). Nevertheless, when inseminated by FTAI, anovular cows had lower P/AI on d 60 than cyclic cows (16.3% [22/135] vs. 25.6 [50/195]). In conclusion, peri-parturient diseases were highly associated with increased anovular condition. Additionally, anovular cows had delayed first postpartum AI and lower P/AI when submitted to FTAI.

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Key Words: disease, estrus, fertility.

1075 Increasing fatty acid oxidation improves insulin sensitivity in primary differentiated bovine adipocytes. J. E. Rico*, F. Seck, M. V. Pinti, J. W. McFadden, *West Virginia University, Morgantown, WV*

Dairy cows develop insulin resistance during the transition from gestation to lactation. Because insulin is an anti-lipolytic hormone, insulin resistance promotes adipose tissue lipolysis. Increasing fatty acid oxidation (FAox) is a means to improve insulin sensitivity in monogastrics. Therefore, our objective was to evaluate the effects of a pharmacological stimulator of FAox on insulin sensitivity in bovine adipocytes. To test our objective, we utilized subcutaneous adipose tissue collected from Angus steers. Stromal-vascular cells were grown from explants in DMEM/F12 growth medium containing 17.5 mM glucose and 10% fetal bovine serum (FBS). Cells were harvested by trypsinization and replated. Once confluent, cells were differentiated using DMEM/F12 medium containing 17.5 mM glucose, 5 mM acetate, 1 mM octanoate, 5% FBS, 1.72 mM insulin, 0.25 mM dexamethasone, 0.5 mM isobutylmethylxanthine, and 2 mM rosiglitazone. Following d 8 of differentiation, cells were cultured in DMEM/F12 treatment medium containing 5 mM glucose, 1 mM acetate, 1% FBS, and 1.2 nM insulin. Differentiated adipocytes were treated with C75 (stimulator of carnitine palmitoyltranserase 1 and FAox; 0 to 100 μM) or palmitic acid (C16:0; 0 to 800 μM) complexed with bovine serum albumin for 3 to 18 h. Control cells did not receive C75 or C16:0 treatment. A minimum of two independent experiments with three replicates per experiment were performed. The statistical model included the fixed effects of treatment, experiment, and their interaction. Replicate within experiment was the random effect. Triacylglycerol (TAG) accumulation and cell viability were determined using colorimetric and fluorescence assays, respectively. Measurement of FAox and insulin sensitivity were measured using radiolabeled 2-deoxyglucose (2DOG) and C16:0. Relative to undifferentiated adipocytes, TAG accumulation was 736% greater in differentiated adipocytes ($P < 0.01$). Following differentiation, treatment with C16:0 or C75 for 18 h did not impair cell viability. Interestingly, 100 μM C75 improved cell viability by 40%, relative to control ($P < 0.01$). Treatment of adipocytes with 100 μM C75 for 3 h increased FAox, as demonstrated by a 122% increase in the recovery of radiolabeled acid-soluble products as well as a 30% increase in radiolabeled CO_2 ($P < 0.05$). Although C16:0 did not modify insulin 2DOG uptake following an 18 h treatment, treating adipocytes with 100 μM C75 for 18 h increased 2DOG uptake by 141%, relative to control ($P < 0.01$). We conclude that the stimulation of FAox enhances insulin-stimulated glucose uptake in primary differentiated bovine adipocytes.

Key Words: C75, fatty acid oxidation, insulin sensitivity

1076 Global gene expression in the endometrium of primiparous dairy cows during the early-luteal phase of the estrous cycle. A. L. Astessiano Dickson*[1], F. Peñagaricano[2], A. Meikle[3], and M. Carriquiry[1], *[1]Facultad de Agronomia, Universidad de la Republica, Montevideo, Uruguay, [2]University of Florida, Gainesville, [3]Facultad de Veterinaria, Montevideo, Uruguay.*

The uterine endometrium plays a central role in early conceptus-maternal communication for the establishment and maintenance of pregnancy. Primiparous Holstein dairy cows were used in a randomized block design to evaluate gene expression changes in the endometrium during the early-luteal phase of the estrous cycle induced by two different feeding strategies: total mixed ration (TMR) vs. pasture + TMR applied during early lactation. In particular, during the first 65 d of lactation, cows were fed either [i] TMR ad libitum (17 kgDM/d offered; 70% forage, 30% concentrate; T1) or [ii] grazing of alfalfa (*Medicago sativa*; 6-h am grazing in 3-d strips; pasture allowance = 20 kgDM/d) plus TMR (70% of ad libitum TMR; 12 kgDM/d offered; T2). At 45 ± 1 d, cows were synchronized and at d 7 of the estrous cycle (d 0 = estrous) endometrial biopsies were obtained. A total of 10 endometrium samples (5 cows per treatment) were analyzed using RNA sequencing. Sequence reads were mapped to the bovine reference genome (bosTau7) using Tophat and the resulting alignments were used to reconstruct transcript models using Cufflinks. Gene expression differences were tested using edgeR package. From the 14,753 genes detected

in the transcriptome, 102 genes were differentially expressed (FDR = 0.10; fold change $\geq$ 2) between T1 vs. T2. Specifically, 20 genes were significantly upregulated in T1 while 82 genes were upregulated in T2. Many of these genes are involved in biological processes such as regulating enzymatic activity (e.g., phospholamban, secretoglobin family 1A member 1), protein binding (e.g., caveolin 3, α-2-HS-glycoprotein), and immune response (e.g., immunoglobulin heavy constant epsilon, major histocompatibility complex class II DQ β, myelin protein P0-like). Functional enrichment analysis, using both Gene Ontology and KEGG databases, revealed significant terms associated with cell and tissue development, cell adhesion, endopeptidases, calcium ion transport, calcium signaling, tryptophan metabolism, among others, with most of the genes being upregulated in T2 compared to T1. Overall, this study characterized genes and pathways expressed in the endometrium of dairy cows at Day 7 of the estrous cycle, and evidenced a differential endometrial environment according with a nutritional management during early lactation in which most of the genes differentially expressed were upregulated in grazing cows.

Key Words: dairy cows, nutrition, transcriptome

1077 Influence of reproductive indicators and genetic parameters on lactation curves.

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Low levels of reproduction indicators in dairy farms are linked to low net returns associated with low milk production and replacement levels and high breeding and veterinary costs. The objectives of this study were to assess the association between lactation curve and reproductive efficiency and to evaluate the genetic variability. A novel reproductive indicator that combines pregnancy status after first (Prg first AI) and second artificial insemination (Prg second AI) and subsequent pregnancy loss after first (Non-Prg first AI) and second artificial insemination (Non-Preg second AI) was developed. The lactation curve was described using Wilmink's and Wood's functions that were incorporated into nonlinear mixed effects models that included the effect of sire, contemporary group, lactation number, the novel reproductive indicator, months open after calving, and cyclicity status at Day 35 after calving. Data on more than 50,000 test-day milk records from more than 6000 U.S. Holstein cows were considered. Cyclicity status was not associated with any lactation curve parameter. Higher months open was associated with higher persistency in milk yield. Estimates from the Wilmink function indicated that cows positive for Prg first AI and negative for Non-Prg first AI and cows negative for Prg first AI, positive for Prg second AI, and negative for Non-Prg second AI had significantly higher levels of milk yield during lactation, higher milk yield at peak production, and lower persistency than cows positive for Non-Prg first and 2ndAI. The Wood's parameter estimates confirmed the higher milk yield immediately after calving, higher incline in milk yield after calving, and higher persistency of the former relative to the latter reproductive groups. The ratio of sire to residual variance estimates was 0.4 and was consistent across models. The novel reproductive indicator that integrates reproduction-related variables intrinsic to the cow combined information on pregnancy and pregnancy loss at the first two AI events and together with genetic parameter estimates offers insights into the factors influencing the lactation curve. These findings contribute to a long-term multistate project database (USDA-NIFA-AFRI-003542) for direct measures of fertility.

Key Words: lactation curves, nonlinear mixed models, reproductive efficiency

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1078 Hematocrit, milk yield, and production related parameters comparisons between slick and wild-type-haired Puerto Rican Holstein cows.

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Previous research has suggested the presence of a slick hair coat as well as greater hematocrit values (HCT) as adaptations resulting in the thermoregulatory superiority of *Bos indicus* in comparison with *Bos Taurus* cattle. However, although it is well established that wild-type-haired (WT) Holstein cows have inferior thermoregulatory capacity than their slick-haired (SLICK) counterparts, there has been no research comparing both phenotypes in terms of HCT values in the Puerto Rican dairy cattle population. Therefore, the present study compared the HCT values (recorded in triplicate) from 29 SLICK and 35 WT lactating Puerto Rican Holstein cows. The SLICK and WT classifications were previously confirmed in a genotyping experiment. Additionally, to determine the uniformity of the evaluated groups the body weight (BW), days in milk (DIM), number of lactations, and production (averaged from the week

before the sampling) were compared between phenotypes. Data, averaged by cow, were analyzed using the GLIMMIX procedure of SAS. No differences were found in BW (538.25 ± 11.77 vs. 570.31 ± 15.61kg; $P = 0.5098$), DIM (187.57 ± 16.25 vs. 186.90 ± 18.17; $P = 0.9992$), and lactation number (1.94 ± 0.25 vs. 2.17 ± 0.22; $P = 0.9984$) between WT and SLICK cows, respectively. However, WT cows exhibited lower milk production values than their SLICK counterparts (17.11 ± 0.63 vs. 20.26 ± 1.28 kg/d; $P = 0.0288$, respectively). Nevertheless, there were no differences ($P = 0.4040$) in HCT values between the WT and SLICK cows (29.30 ± 0.46 vs. 29.79 ± 0.49, respectively). Although there was a difference in milk production, our results suggest that greater HCT values are not part of the adaptations to hot environments present in the Puerto Rican slick-haired Holstein cattle.

Key Words: heat stress, hematocrit, milk yield, slick-haired

1079 Effect of milk yield genotype on hepatic metabolic gene expression and repeated lipopolysaccharide (LPS) administration. G. T. Cousillas[*1], W. J. Weber[1], B. Walcheck[1], R. Chebel[1], D. E. Kerr[2], T. H. Elsasser[3], and B. A. Crooker[1], *[1]University of Minnesota, Saint Paul, [2]University of Vermont, Burlington, [3]USDA, Agricultural Research Service, Beltsville, MD.*

Objectives were to determine effects of milk yield genotype on hepatic expression of genes related to the somatotropic axis and glucose and lipid metabolism during an LPS challenge. Multiparous cows ($n = 12$/genotype) from unselected (stable milk yield since 1964, UH) and contemporary (CH) Holsteins that differed by more than 4500 kg milk/305-d were housed together and fed the same diet ad libitum for more than 4 mo before being blocked (2/genotype) by DIM and randomly assigned within genotype to receive saline or 0.25 μg/kg BW of LPS (*Escherichia coli* 055:B5). Cows were synchronized to be at Day 8 of their estrous cycle for the first challenge (C1) at 70–84 DIM. Liver biopsies were collected at 0, 4, and 24 h relative to treatment. A second identical challenge (C2) and sampling was conducted 4 d later. RNA was extracted and expression of 23 genes associated with metabolism were determined by digital multiplexed analysis (nanoString nCounter). Expression was normalized to the positive control and the geometric mean of 4 internal control genes. Data were transformed (square root) and analyzed by repeated measures using PROC MIXED (SAS) with time as the repeated effect. Means differed when $P < 0.05$. Sixteen genes presented a time by treatment interaction due to changes in expression after LPS. Expression of INSR, INSR-b, and IGFBP2 was greater and GHR-1A was less in CH than UH, expression of these genes decreased in response to LPS in both challenges, but the response did not differ between genotypes. There was a time by challenge interaction for IGFBP2 as it was decreased at 24 h in C1 but not in C2. There were challenge effects for IGF1 and GHR-1A due to greater expression in C1 than in C2 but the response did not differ between genotypes. There were time by treatment interactions for PC1, PCK1, PPARa, and PPARd. In response to LPS expression of PC1 and PPARd increased, and PCK1 and PPARa decreased at 4 h in both challenges, but the response did not differ between genotypes. Results indicate that LPS administration altered hepatic expression of genes related to the somatotropic axis, glucose, and lipid metabolism and that these responses were similar for the low and high milk yield genotypes.

Key Words: bovine genotype, gene expression, lipopolysaccharide, metabolism

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1080 Milk yield genotype impacts expression of hepatic innate immune genes during the transition period in Holsteins. *G. T. Cousillas[*1], W. J. Weber[1], B. Walcheck[1], D. E. Kerr[2], T. H. Elsasser[3], and B. A. Crooker[1], [1]University of Minnesota, Saint Paul, [2]University of Vermont, Burlington, [3]USDA, Agricultural Research Service, Beltsville, MD.*

Objectives were to determine effects of milk yield genotype on hepatic expression of genes related to innate immunity. Multiparous cows from unselected (stable milk yield since 1964; UH; $n = 10$) and contemporary (CH; $n = 10$) Holsteins that differed in milk yield by more than 4500 kg milk/305-d were housed together, fed the same diet ad libitum, and milked 2X/d. Liver biopsies were collected at −14, 3, 14, and 42 d in milk (DIM). RNA was extracted and expression of 44 genes was determined by digital multiplexed analysis (nanoString nCounter). Expression was normalized to the positive control and the geometric mean of 4 internal control genes. Data were transformed (square root) and analyzed by repeated measures using PROC MIXED (SAS) with DIM as the repeated effect. Means differed when $P < 0.05$. Expression of 19 genes was altered by DIM. Expression of 9 genes was greater and 9 genes was less in CH than UH. There were genotype by DIM interactions for CD14 and C/EBPd. Expression of CD14 was lower in CH than UH. In both genotypes, expression of CD14 decreased at 3 DIM, but remained lower in CH through 42 DIM, while CD14 expression recovered at 14 DIM in UH. C/EBPd expression was greater in CH than UH. In CH expression of C/EBPd increased at 3 DIM and returned to prepartum values at 42 DIM. Expression of C/EBPd in UH did not decrease until 42 DIM. LBP and XBP1 were greater in CH than UH, increased at 3 DIM and recovered by 14 DIM. TLR4 decreased at 3 DIM and although it was increasing, remained less than prepartum expression through 42 DIM for both genotypes. TLR2 and ICAM1 were less in UH than CH, decreased at 3 DIM and remained decreased by 42 DIM. XDH was greater in CH than UH, increased at 14 DIM and remained increased by 42 DIM. IL-1b was greater in UH than CH, but its receptor (IL1bR1) was less in UH than CH

and there was no effect of DIM. Results indicate expression of genes involved with cytokine production, inflammation, cell differentiation and activation were altered in both genotypes during the transition period and that there was a less robust response in the contemporary cow.

Key Words: gene expression, Holstein genotype, innate immunity

1081 Effect of milk yield genotype on hepatic metabolic gene expression during the transition period.

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Objectives were to determine effects of milk yield genotype on hepatic expression of genes related to the somatotropic axis and carbohydrate and lipid metabolism. Multiparous cows from unselected (stable milk yield since 1964; UH; $n = 10$) and contemporary (CH; $n = 10$) Holsteins that differed in milk yield by more than 4500 kg milk/305-d were housed together, fed the same diet ad libitum, and milked 2X/d. Liver biopsies were collected at −14, 3, 14, and 42 d in milk (DIM). RNA was extracted and expression of 23 genes was determined by digital multiplexed analysis (nanoString nCounter). Expression was normalized to the positive control and the geometric mean of 4 internal control genes. Data were transformed (square root) and analyzed by repeated measures using PROC MIXED (SAS) with DIM as the repeated effect. Means differed when $P < 0.05$. Expression of 17 genes was altered by DIM. Liver expression of 8 genes was greater and 5 genes was less in CH than UH. There was a genotype by DIM interaction for STAT5A as it decreased at 3 DIM and recovered by 42 DIM in CH but did not change in UH. GHR-1A and IGF-1 were less and STAT3 greater in CH than UH but expression of JAK2 and STAT5B did not differ. There was a genotype by DIM interaction for PC1 as it increased at 3 DIM in both genotypes and remained increased by 42 DIM in CH; in UH PC1 returned to prepartum values at 42 DIM. INSR, PCK1, and PPARGC1A were greater in CH than UH, increased at 3 DIM and remained increased by 42 DIM. PPARa did not differ between genotypes, decreased at 3 DIM and returned to pre-partum values by 14 DIM. PPARd did not differ between genotypes or DIM. Results are consistent with postpartum reduction in hepatic sensitivity to somatotropin which lasted longer in CH cows. During the postpartum interval, expression of INSR and genes for enzymes related to gluconeogenesis were greater in CH than UH which is consistent with their greater need for lactose synthesis in the contemporary cow.

Key Words: gene expression, milk genotype, transition

1082 Gene expression and secretion of chemerin in bovine mammary epithelial cells.

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A variety of cytokines are secreted in a paracrine manner within the bovine mammary gland to maintain the microenvironment of the tissue. Our previous study showed that treatment with chemerin induced the expression of genes related to lactogenesis in immortalized bovine mammary epithelial cells (MAC-T cells). This suggested that chemerin is a secreted protein with chemotactic ability to antigen presenting cells has a regulatory effect on the function of mammary gland. However, what type of cell in mammary glands expresses chemerin and what kind of factor regulates its expression are not clear. In this study, we investigated the chemerin protein expression in bovine mammary tissues, milk, and cultured MAC-T cells. Mammary tissues were sampled from Holstein dairy cows in the lactation and dry-off periods, and chemerin protein expression was determined immunohistochemically. Chemerin protein was also detected in fresh milk and cell lysate of MAC-T cells by western blotting. Further, the effect of TNF-α on chemerin mRNA expression was investigated in MAC-T cells. MAC-T cells were grown until confluence and then treated with TNF-α (0.1, 1, and 10 nM) for 24 h. Chemerin gene expression was analyzed by Q-RT-PCR. Statistical analysis was performed using Tukey's HSD test. The results showed that chemerin protein was expressed in epithelial cells and stromal cells of bovine mammary gland from Holstein dairy cows and in cultured MAC-T cells. In addition, secreted chemerin protein was also detected in fresh milk. TNF-α significantly induced chemerin mRNA expression in MAC-T cells ($p < 0.05$). These results indicate that chemerin is secreted within the mammary gland in an auto/paracrine manner and that chemerin expression is upregulated by inflammatory cytokine. This study suggests the roles of chemerin as a chemoattractant that might attract immune cells to eliminate infectious bacteria or apoptotic cells in the mammary gland, as well as its role in lactogenesis.

Key Words: chemerin, inflammatory cytokine, mammary epithelial cells

1083 Proteomic analysis reveals increased Nrf2-mediated oxidative stress response in adipose tissue of late pregnant dairy cows during summer heat stress. M. Zachut[*1], G. Kra[1], G. Friedlander[2], and Y. Levin[3], *[1]Institute of Animal Science, Volcani Center, Bet Dagan, Israel, [2]The Ilana and Pascal Mantoux Institute for Bioinformatics, Weizmann Institute of Science, Rehovot, Israel, [3]The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot, Israel.*

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a critical regulator of key aspects of the antioxidant defense pathway under chronic stress. Oxidative stress and Nrf2 affect adipose tissue (AT) function. Heat stress at late pregnancy affects the physiology and performance in subsequent lactation. We investigated the effects of seasonal heat load on the proteome of adipose tissue in late pregnant dairy cows. Adipose tissue biopsies were obtained from 18 multiparous late pregnant dry cows at 14 d before expected calving during summer (S, n = 10) or winter (W, n = 8). Cows were also divided retrospectively according to BW loss during the first month postpartum to HWL–high weight loss (n = 9), and LWL–low weight loss (n = 9). Blood samples were collected twice a week for oxidative stress marker malondialdehyde (MDA) and cortisol concentrations. Proteins were analyzed by intensity based, label-free quantitative shotgun proteomics at Weizmann Institute of Science (Rehovot, Israel). Proteins were extracted and subjected to in-solution tryptic digestion, followed by nanoflow liquid chromatography coupled to high-resolution tandem mass spectrometry (nanoLC-MS/MS). Quantitative data were extracted using Genedata Expressionist data analysis package and proteins identified using Mascot search engine. Proteomics data, after logarithmic transformation, were analyzed by two-way ANOVA for effects of season (S vs. W), subgroup (HWL vs. LWL), and their interaction. Both pre- and postpartum, S cows had higher plasma MDA and cortisol concentrations compared with W (P < 0.005). Proteomic analysis quantified 1496 proteins in AT, from which the abundance of 132 (8.8%) proteins was differential in S vs. W [Fold change (FC) ± 1.5 and P < 0.05]. One of the top canonical pathways affected by season was Nrf2-mediated oxidative stress response (Ingenuity Pathway Analysis, Qiagen); the abundance of ubiquitin-conjugating enzyme E2 K (P < 0.006) and stress-induced-phosphoprotein 1 (P < 0.001) was elevated, while mitogen-activated protein kinase kinase 1 (P < 0.02), ferritin (P < 0.02), glutathione S-transferase Mu 1 (P < 0.04) and microsomal glutathione S-transferase 1 (P < 0.02) decreased in S vs. W adipose. These findings imply that Nrf2-mediated oxidative stress response plays a main role in the reaction of AT to counterbalance the increased oxidative stress under heat stress conditions in late pregnant dairy cows.

Key Words: adipose, heat stress, proteomics, transition

1084 Cholesterol deficiency associated APOB mutation affects lipid metabolism in Holstein cattle. J. J. Gross[*1], A. C. Schwinn[1], F. Schmitz-Hsu[2], F. Menzi[3], C. Drögemüller[3], C. Albrecht[4], and R. M. Bruckmaier[1], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Swissgenetics, Zollikofen, Switzerland, [3]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [4]Institute of Biochemistry and Molecular Medicine, University of Bern, Bern, Switzerland.*

During the last months, the number of reports on Holstein calves suffering from incurable idiopathic diarrhea dramatically increased. Affected calves showed severe hypocholesterolemia, and mostly died within days up to a few months after birth. This new autosomal monogenic recessive inherited fat metabolism disorder termed cholesterol deficiency (CD) is caused by a loss of function mutation of the bovine *APOB* gene. The objective of the present study was to investigate specific components of the lipid metabolism in 6 CD affected homozygous for the *APOB* mutation (CDS) and six normal Holstein calves with different *APOB* genotypes. Independent of sex, CD affected calves (CDS) had significantly lower plasma concentrations of total cholesterol (TC), free-cholesterol (FC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), triacylglycerides (TAG), and phospholipids (PL) compared to homozygous wild-type calves (P < 0.05). Furthermore, we studied the effect of the *APOB* genotype on cholesterol metabolism in adult Holstein breeding bulls of Swissgenetics. Among a total of 254 adult males the homozygous mutant genotype was absent, 36 bulls were heterozygous carriers (CDC), and 218 homozygous wild-type (CDF). In CDC bulls, plasma concentrations of TC, FC, HDL-C, LDL-C, VLDL-C, TAG, and PL were lower compared to CDF bulls (P < 0.05). The ratios of FC:cholesteryl esters (CE), and FC:TC were higher in CDC compared to CDF bulls, whereas the ratio of CE:TC was lower in CDC compared to CDF bulls (P < 0.01). In conclusion, the cholesterol deficiency associated *APOB* mutation was shown to affect lipid metabolism in affected Holstein calves and adult breeding bulls. Besides cholesterol also the concentrations of PL, TAG, and lipoproteins were distinctly reduced in homozygous and heterozygous carriers of the *APOB* mutation. Beyond malabsorption of dietary lipids, deleterious effects of apoB deficiency on hepatic lipid metabolism, steroid biosynthesis, and cell membrane function can be expected, which may result in unspecific symptoms of reduced fertility, growth, and health.

Key Words: cholesterol deficiency, hypobetalipoproteinemia, hypocholesterolemia

1085 Characterization of changes in temporal concentrations of fibroblast growth factor 21 (FGF21) before and after parturition in multiparous beef cows. L. Prezotto*[1], J. F. Thorson[1], J. Dafoe[1], M. R. Herrygers[2], and J. G. Berardinelli[2], *[1]Montana State University, Havre, [2]Montana State University, Bozeman.*

The objectives of this experiment were to characterize the secretion of Fibroblast Growth Factor 21 (FGF21) in beef cows during the last month of gestation, parturition, and early lactation and correlate these concentrations with metabolites. Pregnant, multiparous cows (n = 30) fed a TMR to meet or exceed NRC requirements were weighed and blood samples collected on days −14, −7, 0, 14, 28, and 60 relative to parturition. Samples were assayed for concentrations of FGF21, glucose, BUN, and NEFA. Individual average daily gain (ADG) was calculated for the experimental period. As previously shown in dairy cows, concentrations of FGF21 increased as parturition neared with concentrations of FGF21 increasing (P = 0.003) from day −14 (447 ± 120 pg/ml; mean ± SE) to 0 (790 ± 87 pg/ml). After parturition, concentrations of FGF21 decreased (P < 0.0001) by Day 14 (299 ± 88 pg/ml) to concentrations no different than day −14. Concentrations of FGF21 were maintained (P = 0.85) after parturition to Day 28 (288 ± 88 pg/ml). At Day 60, concentrations of FGF21 tended (P = 0.08; 391 ± 87 pg/ml) to increase compared to concentrations on Day 28. Concentrations of FGF21 and ADG tended (P = 0.09) to be negatively correlated on Day 56. Concentrations of glucose increased (P = 0.002) between day −14 and 0 and then decreased (P = 0.04) between Day 0 and 14. Concentrations of BUN increased (P = 0.0006) from day −14 to 0, decreased (P = 0.03) between Day 0 and 14, then continued to decrease (P < 0.0001) from Day 28 to 60. Finally, concentrations of NEFA decreased (P < 0.0001) from day −14 to 0 then increased (P = 0.04) by Day 14 to concentrations maintained (P = 0.94) to Day 28. There was no correlation (P ≥ 0.42) between metabolites and FGF21 on Day 0. However, on Day 60 concentrations of FGF21 tended (P = 0.09) to be negatively correlated with concentrations of glucose, positively correlated with concentrations of BUN (P = 0.0004), and not correlated with concentrations of NEFA (P = 0.30). These data indicate that FGF21 can be used as a biomarker to indicate reproductive and nutritional status in the beef cow.

Key Words: metabolites, parturition, performance

1086 Effect of investigational kisspeptin/metastin analog, TAK-683, on luteinizing hormone secretion at different stages of the luteal phase in goats. L. P. Rahayu*[1,2], M. E. Behiry[3], N. Endo[1,2], and T. Tanaka[1,2], *[1]Tokyo University of Agriculture and Technology, Tokyo, Japan, [2]United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan, [3]Visiting Research Scientist from Egypt, Tokyo University of Agriculture and Technology, Tokyo, Japan.*

Our previous study showed differential changes in luteinizing hormone (LH) secretion after administration of an investigational kisspeptin/metastin analog, TAK-683, between the endocrine conditions of the follicular and luteal phases in goats (Anim. Reprod. Sci. 2015). The present study aimed to examine the response of LH secretion to TAK-683 treatment and its association with ovarian changes during different stages of the luteal phase in goats. Nine cycling Shiba goats (4.4 ± 2.3 yr old) were assigned into 3 groups: early luteal phase (ELP, n = 4), mid-luteal phase (MLP, n = 4), and a control (n = 5) group. The ELP and MLP groups were administered 50 µg of TAK-683 intravenously on the 5 d after ovulation and on Days 7–14 after ovulation, respectively. The control group received vehicle on Days 7–14 after ovulation. Blood samples were collected at 10-min (hours 2–6) or 2 h (hours 6–24) intervals, and at 48 h after treatment for analysis of endocrine profiles. Ovarian ultrasonographic images and estrous behavior were assessed daily or every other day until the subsequent ovulation for analysis of effects on follicular and luteal dynamics and the length of the estrous cycle. LH concentration increased with a relatively small amplitude within 6 h of treatment with a mean peak value of 1.9 ± 0.6 ng/ml in the MLP group. Meanwhile, in the ELP group, TAK-683 treatment initially induced a sustained rise in the LH concentration with a relatively small amplitude, and then a surge-like release of LH with the highest values of 18.4 and 12.8 ng/ml, and with a peak time at 12 h and 14 h after treatment, respectively, in 2 goats. There is no significant difference in the progesterone concentration between pre- and post-treatment periods in all groups. Ovulation was detected within 2 d of the administration of TAK-683 in 2 goats showing a surge-like release of LH. Time-course of the LH response to TAK-683 treatment in the 2 goats showing a surge-like release of LH in the ELP group support our previous study that initial secretory response of LH to TAK-683 treatment is characterized as a small amplitude increase, and the secretion pattern of LH subsequently changes to a robust increase. It is suggested that responses of pulsatile and surge mode secretions of LH to the treatment of a kisspeptin analog depend on the stage of luteal phase in cycling goats.

Key Words: kisspeptin, luteal phase, luteinizing hormone

1087 MAC-T cell as in vitro evaluation system for casein gene expression involving glucose level.
H. Y. Jeong*[1], Y. T. Heo[2], H. S. Kang[1], E. T. Kim[1], and H. Song[2], *[1]Dairy Science Division, National Institute of Animal Science, RDA, Cheonan-si, Korea, [2]Konkuk University, Seoul, Korea.*

Glucose is essential fuel in energy metabolism and synthesis pathways of all mammalian cells. In lactating animals, glucose is the major precursor for lactose and is a substrate for the synthesis of milk proteins and fat in mammary secretory (alveolar) epithelial cells. However, clear utilization level of glucose in mammary cell during lactogenesis is still unknown due to lack of in vitro analyzing model. Therefore, objective of this study is to test reliability of mammary alveolar (MAC-T) cell as in vitro study model for glucose metabolism and lactating system. Undifferentiated MAC-T cells were cultured in three types (Non-glucose: 0 g/L, low glucose: 1 g/L, and High glucose: 4.5 g/L) of DMEM for 8 days, and then differentiation was induced. Cell proliferation and expression levels of apoptotic genes, IGF1 receptor, Oxytocin receptor, αS1, αS2, and β casein genes were analyzed at 1, 2, 4, and 8 d after differentiation. Proliferation of MAC-T cells with high glucose treatment was significantly higher. Expression of apoptotic genes was not affected by any groups. However, expression levels of mammary development related gene IFG1 r and lactating related gene Oxytocin r were significantly higher in low glucose group. Expressions of αS1-casein, αS2-casein, and β-casein were also higher in low glucose treated group compared with none and high glucose groups. Results demonstrated that although high glucose environment to MAC-T cells increase cell proliferation, low glucose treatment to MAC-T cells induce higher expression of casein genes. Our results may suggest that MAC-T cell can be in vitro model to analyze mammary cell development and lactation connect with precise biological effects.

Key Words: casein, glucose, IGF1, mammary alveolar (MAC-T) cell, oxytocin

1088 mRNA abundance of steroid hormone metabolizing enzymes (17β-HSD isoforms and CYP19) in adipose tissue of dairy cows during the periparturient period.
A. Alizadeh[1,2,3], H. Sadri[1], J. Rehage[4], S. Dänicke[5], and H. Sauerwein*[1], *[1]Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Bonn, Germany, [2]Department of Animal Science, Saveh Branch, Islamic Azad University, Saveh, Iran, [3]Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran, [4]University for Veterinary Medicine, Foundation, Hannover, Germany, [5]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Braunschweig, Germany.*

Besides being a storage organ for lipophilic compounds such as steroids, adipose tissue (AT) is now recognized in humans as being also capable of synthesizing and interconverting steroid hormones. In view of the comprehensive changes in body fat content of dairy cows during the lactation cycle, effects on steroid metabolism and release and potentially on fertility are conceivable. With this background, our research objectives were (1) to assess the mRNA expression of 17β-hydroxysteroid dehydrogenases (17β-HSD), i.e., the enzymes catalyzing the interconversion between the active and inactive forms of specific steroid hormones (focusing on subtypes 17β-HSD-1, −3 and −12), and of P450-aromatase (CYP19), converting androgens to estrogens, in bovine AT, (2) to characterize the time course of their mRNA abundance during late pregnancy and early lactation and (3) to compare this time course in a subcutaneous versus a visceral fat depot. From 20 Holstein cows, biopsies were collected from the subcutaneous (sc) and the retroperitoneal fat depot (rpAT) on d −42 and d 1, 21, and 100 relative to calving. The mRNA abundance of the target genes was assessed in the tissue samples by qPCR and normalized by using the 4 most stable reference genes. Data were analyzed using the MIXED procedure of SAS. Among the 4 target genes studied, only 17β-HSD-12 mRNA was detectable with the protocol used herein (suitability of the protocols was confirmed using ovary as positive control). The mRNA abundance of 17β-HSD-12 in scAT was highest on d −42, followed by a substantial decline on d 1 and 21 (7.5- and sixfold, respectively), and an increase on d 100 ($P < 0.001$). In rpAT, the peripartal changes of 17β-HSD-12 mRNA abundance were largely analogous to the ones observed in scAT; i.e., the values on d −42 and 100 were greater than on d 1 and 21 ($P < 0.001$). Our results indicate that aromatization of androgens to estrogens via CYP19 is not taking place in bovine AT, but estradiol-17β might be formed in AT by 17β-HSD-12 from estrone taken up from the circulation.

Key Words: 17β-HSD, adipose tissue, dairy cows

1089 Mitochondrial biogenesis and DNA content in metabolically tissues of lactating cows with divergent milk production. R. Weikard* and C. Kühn, *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

An appropriate metabolic adaptation of key tissues important for bioenergetic homeostasis and lactogenesis is required in cows to adjust for changes in energy demands and physiological conditions during the lactation period. Mitochondria are recognized to be central for meeting energy demands and maintaining metabolic homeostasis, and mitochondrial DNA (mtDNA) content reflects the capacity of cells for energy generation. The focus of our study was to elucidate, if mtDNA content and mitochondrial biogenesis were associated with lactation performance of cows characterized by a divergent genetic background regarding milk production. Therefore, we took advantage of cows from a resource population with a combined genetic dairy and beef background (Charolais x German Holstein cross, CHxGH) and compared them to purebred German Holstein (GH) dairy cows regarding mtDNA content and mRNA expression of important nuclear encoded genes controlling mitochondrial biogenesis in in two metabolically active tissues, liver and mammary gland. Genomic DNA and total RNA were isolated from tissue samples of 30 cows separated into three experimental groups based on their milk production during the week before slaughtering (each $n = 10$). Analysis of expression of genes involved in mitochondrial biogenesis, replication/transcription and translation of mtDNA and determination of mtDNA content were performed using quantitative real-time PCR. The study revealed a tissue-specific variation of mtDNA content that is higher in liver than in mammary gland, which agrees to the higher hepatic metabolic activity. When comparing high-lactating GH cows to cows with medium lactation performance (CHxGH-M), the mtDNA content was similar in liver but clearly reduced in mammary gland in GH cows. Unexpectedly, the mtDNA levels in mammary gland of GH cows resembled those of low-lactating cows (CHxGH-L). Gene expression analysis revealed lower transcript levels of genes involved in mitochondrial biogenesis, replication/transcription and translation in the liver of GH cows compared to CHxGH-M cows. In the mammary gland of GH cows, the gene expression levels pointed to a reduced mitochondrial biogenesis and mtDNA translation compared to CHxGH-M cows, whereas transcript levels related to mtDNA transcription/replication did not differ between both cow groups. The results suggest that the hepatic and mammary mitochondrial biogenesis processes are differentially modulated in high-lactating dairy cows and lactating cows from a CHxGH cross population indicating toward impaired mitochondrial biogenesis during high lactation and obviously reflecting genetic differences in coping with metabolic and physiological changes during lactation.

Key Words: gene expression, lactation, liver, mammary gland, mitochondrial DNA, mitochondrial biogenesis

1090 Lipopolysaccharide exposure in swine alters ovarian toll-like receptor 4 expression. K. L. Bidne*, M. J. Dickson, S. K. Kvidera, L. H. Baumgard, J. W. Ross, and A. F. Keating, *Iowa State University, Ames.*

Heat stress (HS) is associated with decreased fertility and endotoxemia as evidenced by increased systemic lipopolysaccharide (LPS) arising from decreased intestinal integrity. Across multiple species, LPS is associated with reduced female fecundity; phenotypic responses similar to HS-induced infertility, including spontaneous abortion and increased wean-to-estrus interval length. LPS binds to toll-like receptor 4 (TLR4), a membrane bound receptor, to initiate a signaling cascade culminating in the phosphorylation of nuclear factor kappa-B (pNFκB) and pro-inflammatory cytokine production. Acyloxyacyl hydrolase (AOAH) participates in LPS detoxification by cleaving the lipid A moiety, rendering the deacylated LPS unable to effectively bind TLR4. We hypothesized that endotoxemia could impact ovarian function in swine. Postpubertal gilts were synchronized to the follicular phase of their estrous cycle using Matrix® administered orally for 14 d. Immediately following Matrix® removal, gilts were treated with vehicle control (CT; 3 mL sterile saline; $n = 6$) or LPS (0.1 μg/kg BW; *E. coli* 055:B5; $n = 6$) via jugular catheter four times daily (0000, 0600, 1200, 1800 h) for 5 d during the follicular phase preceding estrus. Six hours after the final LPS infusion, animals were euthanized and ovaries collected. Whole ovarian protein homogenates were prepared and western blotting performed to quantify abundance of TLR4 and AOAH protein. Relative to CT, ovaries from LPS treated gilts had reduced ($P < 0.05$) abundance of TLR4 protein. No effect ($P > 0.05$) of LPS infusion on AOAH abundance was observed. These data demonstrate that the ovary to be responsive to chronic, low-level LPS exposure, and that endotoxemia potentially contributes to seasonal infertility in swine. Funded by the Global Food Security Consortium.

Key Words: endotoxemia, heat stress, ovary

1091 Milk yield genotype affects hepatic expression of innate immune genes when challenged with lipopolysaccharide (LPS).

1092 WS Mycobacterium avium subspecies paratuberculosis serum lipid profile analysis through Fourier transform ion cyclotron resonance mass spectrometry. A. L. Salazar*[1], J. M. Jarvis[1], N. M. Sudasinghe[1], S. Kumar[1], M. Song[1], J. Stabel[2], T. Thacker[2], S. L. Ivey[1], and T. Schaub[1], *[1]New Mexico State University, Las Cruces, [2]USDA-ARS, Ames, IA.*

Mycobacterium avium subspecies paratuberculosis (MAP) is responsible for Johne's disease (paratuberculosis; paraTB) in bovine which elicits serve enteritis in the lower intestinal tract; similar to Crohn's disease in humans. The objective of our study was to observe lipid changes in serum extracts of cattle infected with MAP using ultra-high resolution mass spectrometry. We hypothesized through the use of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) the identification of unique lipid biomarkers induced by MAP infection will be observed. Field samples from cattle infected with MAP (INF; $n = 10$) were provided by the National Animal Disease Center (NADC). Uninfected serum (SC2012 and SC2015) from cattle with no history of paraTB came from two sources provided by the NADC. Negative controls used to test cross reactivity, with no MAP infection or previous exposure, included serum extracts from cattle challenged with lipopolysaccharide (LPS) and cattle infected with *Mycobacterium bovis* (bTB). Spectral differences were observed in the INF treatment compared to all other samples. Heteroatom class distribution, in positive ion mode, showed higher relative lipid abundance in the INF treatment in O_5Na_1, O_3, O_9Na_1, $O_{10}Na_1$, O_8Na_1, N_1, O_7Na_1, and N_1O_1 compounds whereas $N_1O_8P_1$ and $N_1O_7P_1$, were higher abundance in both control treatment groups. In negative-ion mode, O_3compounds were greater relative abundance in the INF treatment and both control treatments had higher relative abundance of $N_1O_{11}P_1$ and $O_{13}S_1$compounds. Assigned elemental compositions were searched in the Lipidomic Gateway Databased to identify relative abundance of lipid classes. The majority of compounds classified in the glycerophospholipid, polyketide, or sterol class. Bioinformatics showed forty-five unique compounds ($P < 0.05$), twenty-six in positive-ion mode, and nineteen in negative-ion mode, purely present in MAP infected cattle; no cross reactivity observed. The shift in heteroatom class distribution provides specific lipids may be present only in paraTB infected cattle, which is confirmed by the compounds identified solely in MAP infected cattle.

Key Words: Johne's disease, lipidomics, *Mycobacterium avium* subspecies *paratuberculosis*, paratuberculosis, ultra-high resoltuion mass spectrometry

1093 WS Insulin-associated and insulin-independent impacts of β adrenergic agonists and pro-inflammatory cytokines on glucose metabolism in primary rat soleus muscle. C. N. Cadaret*, K. A. Beede, H. E. Riley, and D. T. Yates, *University of Nebraska-Lincoln, Lincoln.*

Recent studies show that catecholamines and pro-inflammatory cytokines may help regulate skeletal muscle growth and metabolism even at sub-stress levels. The objective of this study was to determine the acute effects of β1 and β2-specific adrenergic agonists as well as TNFα and IL-6 on muscle glucose uptake and oxidation under basal and insulin-stimulated conditions. Primary soleus muscle was collected from adult Sprague-Dawley rats, separated tendon-to-tendon into 25–45 mg strips, and incubated in KHB spiked with or without insulin, and/or ractopamine HCl (β1 agonist), zilpaterol HCl (β2 agonist), TNFα, and IL-6. Glucose uptake was determined from cellular content of [^{3}H]-2-deoxyglucose after 20 min. Glucose oxidation of [^{14}C-U] glucose was determined after 2 h. Phospho-Akt/total Akt (p-Akt/Akt) was determined from protein isolated after 1 h. Compared to muscle incubated in un-spiked (basal) media, incubation with insulin increased ($P < 0.05$) glucose uptake by ~47%, glucose oxidation by ~32%, and p-Akt/Akt by ~238%. Muscle incubated with β2 agonist exhibited ~20% less ($P < 0.05$) glucose uptake but ~32% greater ($P < 0.05$) glucose oxidation than basal. Moreover, incubation with β2 agonist+insulin increased ($P < 0.05$) glucose oxidation and p-Akt/Akt over insulin alone. Muscle incubated with β1 agonist did not differ from basal for any output. Likewise, β1 agonist+insulin incubations did not differ from insulin alone. Glucose oxidation was ~23% and ~33% greater ($P < 0.05$), respectively, in muscle incubated with TNFα and IL-6 compared to basal, yet glucose uptake and p-Akt/Akt did not differ. Glucose uptake, glucose oxidation, and p-Akt/Akt were similar among muscle incubated with TNFα+insulin, IL-6+insulin, and insulin alone. In addition, glucose oxidation in muscle incubated with TNFα+insulin and IL-6+insulin did not differ from TNFα alone or IL-6 alone. These results show that acute β2 stimulation had opposite effects on glucose uptake and glucose oxidation in muscle, and that acute β1 stimulation had no evident impact on muscle metabolism. Moreover, β2 stimulation was synergistic with insulin, as glucose oxidation and Akt phosphorylation were greater with the two products together than with either individually. Lastly, acute stimulation with TNFα or IL-6 increased glucose oxidation rates independently of insulin or Akt phosphorylation. Together, our findings demonstrate that adrenergic and inflammatory mediators can have insulin-associated or insulin-independent effects on glucose metabolism and that these

effects may differ for glucose uptake and oxidation.

Key Words: β-agonist, glucose metabolism, stress hormones

1094 WS Relationship between current temperament measures and physiological responses to handling of feedlot cattle. A. F. Williams*[1], J. A. Boles[1], M. R. Herrygers[1], J. G. Berardinelli[1], M. C. Meyers[2], and J. M. Thomson[1], *[1]Montana State University, Bozeman, [2]Idaho State University, Pocatella.*

Interest in beef cattle temperament has increased due to growing consumer awareness of animal welfare. Researchers have defined temperament as behavioral responses to a perceived stressor. Subjective chute scoring has been used by many researchers for temperament, however, the subjectivity and associated variability among observers has been questioned. The most practical objective method of assessing temperament is exit velocity. Corresponding chute side measures to physiological markers are important. Faster exit velocities have been related to both increased cortisol and increased plasma lactate. The objectives of this study were to compare temperament differences between feedlot steers and heifers and to confirm chute side measures relationship to physiological responses to stress. Body temperature, serum and plasma lactate, serum glucose, salivary and serum cortisol concentrations were measured on mixed breed and sex feedlot cattle (n = 197). Fast, medium, and slow classifications were developed from exit velocities. Plasma lactate was significantly different between all classes. Sex had a significant effect on exit velocity and physiological measures. Heifers had higher exit velocities (P = 0.003), plasma lactate concentrations (P = 0.03), and cortisol concentrations (P = 0.001). Simple correlations among these variables indicated body temperature (heifers r = 0.44, P < 0.0001; steers 0.45 P < 0.0001), plasma lactate (heifers r = 0.52 P < 0.0001; steers r = 0.63 P < 0.0001), serum lactate (heifers r = 0.53 P < 0.001; steers r = 0.59 P < 0.001) and glucose (heifers r = 0.54 P < 0.001; steers r = 0.32 P < 0.003) were all correlated to exit velocity in both steers and heifers. Cortisol measures were not correlated to exit velocity in steers but were in heifers. Linear models constructed and evaluated using Akaike information criterion indicated that plasma lactate in combination with body temperature were strong candidates to predict exit velocity. Using the discriminate function analysis, the model categorized fast and slow classifications 69.23% and 61.54% respectively, indicating that in combination with exit velocity simple objective chute side measures of body temperature and plasma lactate can potentially increase accuracy of temperament identification.

Key Words: cortisol, lactate, temperament

1095 Cardiovascular performance of modern swine does not comply with allometric scaling laws. G. van Essen*, *University Medical Center Rotterdam, Rotterdam, Netherlands.*

In view of long-standing concerns about possible consequences regarding the cardiovascular capacity and adaptability of modern pigs we investigated the proportionality and performance of porcine hearts over a wide range of body weights (25– 225 kg), according to allometric scaling laws. Specifically, we tested the hypothesis that both heart mass (HM) and cardiac output (CO) scale with body mass (M) to the power of 0.75 (HM or CO = a.M0.75), stroke volume (SV), and left ventricular end-diastolic volume (LVEDV) to the power of 1.00. For this purpose, 21 Yorkshire x Landrace pigs were instrumented under anesthesia to measure CO and SV and LVEDV. Subsequently, animals were sacrificed and hearts were excised and weighed and tissue samples of the LV anterior myocardial wall analyzed for collagen content. Using a linear mixed model, the scaling coefficients of the relations between M and CO, SV, HM, and LVEDLV were determined. The 95% confidence intervals of the power-coefficient b for HM were 0.67– 0.88, encompassing the predicted value of 0.75, indicating that HM increased proportionally to M. In contrast, the 95% confidence intervals of power-coefficient b for CO amounted 0.40–0.65, thus failing to encompass the predicted value of 0.75. This was principally due to the lack of proper scaling of SV as the confidence interval of 0.52–0.83 failed to encompass the predicted value of 1.0, which in turn appeared to be due to a lack of scaling of LVEDV as its confidence interval of b values amounted 0.57–0.99, thus failing to encompass the predicted value of 1.0. The increase of HM without a proportional increase of LV volume, was accompanied by a doubling of collagen content in the LV of swine > 150 kg compared to swine < 75 kg (p < 0.05). In conclusion, cardiac geometry and function of modern swine fail to obey allometric scaling laws, likely due to an increased extracellular matrix deposition preventing physiological remodeling during growth.

Key Words: allometric scaling laws, cardiovascular system, swine

1096 DL-methionine increases glutathione concentration and alleviates inflammatory responses in primary bovine hepatocytes. Q. Zhang*[1], D. N. Luchini[2], and H. M. White[1], *[1]University of Wisconsin-Madison, Madison, [2]Adisseo S.A.S., Alpharetta, GA.*

Supplementation of rumen-protected methionine (Met) to dairy cows during the peripartal period improves postpartal performance and may decrease oxidative stress. The aim of the present study was to determine the effects of increasing concentrations of DL-Met on hepatic inflammatory responses and oxidative status. Hepatocytes isolated from 4 calves less

than 7 d old were maintained as monolayer cultures for 24 h before addition of treatments. Treatments included 0, 10, or 40 μM DL-Met added to Met-free media containing 100 μM Lys (0MET100LYS, 10MET100LYS, or 40MET100LYS), and 10 μM DL-Met added to Met-free media containing 25 μM Lys (10MET25LYS). Both 40MET100LYS and 10MET25LYS had a Met:Lys of 1:2.5. Cells were exposed to each treatment in triplicate for 16 h and then challenged with either 0 or 100 ng/mL lipopolysaccharide (LPS) for 8 h. Cell lysates were collected for quantification of glutathione (GSH) by fluorometic assay and quantification of gene expression by quantitative PCR. Abundance of mRNA was normalized to the mean of 3 reference genes. Cell media was collected for quantification of reactive oxygen species (ROS) by fluorometric assay. Data were analyzed using PROC MIXED of SAS 9.3. The model included treatment, LPS, their interaction, and random effect of calf. Data are reported as LSMEANS ± SE. There was an interaction ($P < 0.05$) of treatment and LPS on GSH concentration which increased ($P < 0.01$) as Met concentration increased (107.5, 114. 5 vs. 131. 5 ± 23.5 μM) without LPS challenge, and 40MET100LYS had greater ($P < 0.01$) GSH than 10MET-25LYS (131. 5 vs. 97.5 ± 23.5 μM). With LPS challenge, GSH concentration was not different ($P > 0.10$) among treatments. Hepatocytes challenged by LPS showed an inflammatory response with increased ($P < 0.001$) expression of tumor necrosis factor (1.425 vs. 2.257 ± 0.344 arbitrary unit (AU)) independent of treatment. However, there was an interaction ($P < 0.01$) of treatment and LPS on interleukin *(IL)-6* expression, which was increased by LPS in cells receiving 10MET-100LYS (1.086 vs. 3.851 ± 0.643 AU) and 10MET25LYS (0.918 vs. 2.296 ± 0.643 AU), but was not increased by LPS in cells receiving 40MET100LYS (0.912 vs. 1.770 ± 0.643 AU). Cell culture media ROS concentration was not different ($P > 0.10$) among treatments with or without LPS. The data suggest that a stress model can be established using primary bovine hepatocytes with LPS challenge. Increasing Met concentration enhances intracellular antioxidant production and alleviates inflammatory responses, although ROS released from the cells was not affected. The treatment effects were attributed to increase in Met concentration, not the Met:Lys.

Key Words: glutathione, interleukin, lipopolysaccharide

1097 Elevated hepatic lipid peroxidation and oxidative stress in underperforming piglets. *T. G. Ramsay*, M. J. Stoll, L. A. Blomberg, and T. J. Caperna, USDA, ARS, BARC, Beltsville, MD.*

The present study was designed to determine if normal weight pigs that grow poorly during the pre-weaning period have altered hepatic metabolism, as previously reported for intrauterine growth retarded pigs relative to littermates with normal growth rates. Eight pairs of average birth weight pigs (1.57 ± 0.05 kg) were identified that diverged in weight by a minimum of 50 g/day until 21 d of age. At 21 d, slow growing (SG) pigs weighed 5.47 ± 0.22 kg while control littermates weight 6.98 ± 0.28 kg ($P < 0.001$). Livers were collected for analysis at Day 21 for analysis of enzyme activity, glycogen content, and gene expression. Metabolomic analysis of the liver tissue was performed by Metabolon (Durham, NC). No changes with growth rate were detected in liver enzyme activity per mg tissue protein for enzymes in glycolysis, lipogenesis, or the pentose phosphate shunt ($P > 0.05$). Liver glycogen content (mg/gm liver protein) was similar between SG piglets and control littermates ($P = 0.908$). The mRNA abundance for the two genes regulating peroxisomal fatty acid oxidation: acyl CoA oxidase 1 (ACOX1; $P < 0.001$) and peroxisome proliferator-activated receptor α (PPARα; $P < = 0.003$), superoxide dismutase 2 ($P = 0.021$), lactate dehydrogenase ($P = 0.016$), insulin-like growth factor 2 (IGF2; $P = 0.002$), IGF binding protein 2 (IGFBP2; $P = 0.004$), and IGFBP3 ($P = 0.015$) were increased in the liver of the SG piglet relative to liver of piglets experiencing normal growth, as measured by real-time quantitative PCR. The increases in PPARα and ACOX1 mRNA abundance suggest that the liver of the SG piglet has the capacity to oxidize an increased proportion of long chain fatty acids relative to the control piglet through peroxisomal β-oxidation. The parallel increase in SOD2 mRNA abundance suggests that SOD2 may function to reduce the oxidative stress caused by an increase in peroxisomal β-oxidation. Metabolomic analysis of the liver from these SG piglets confirmed an increase in mono- and dihydroxy-fatty acids indicative of increased lipid peroxidation and oxidative stress relative to liver from control littermates ($p < 0.05$). These data indicate that the SG piglet utilizes alternative pathways for fatty acid oxidation during the preweaning period which may be a predictor for poor postnatal growth or a target for treatment to improve growth.

Key Words: growth, lipid peroxidation, liver, oxidative stress, pig,

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1098 Yeast supplementation altered the metabolic response to a combined viral-bacterial challenge in feedlot heifers. A. B. Word[*1], P. R. Broadway[2], N. C. Burdick Sanchez[2], K. P. Sharon[3], S. L. Roberts[4], J. T. Richeson[4], P. J. Defoor[5], M. D. Cravey[6], J. R. Corley[7], M. A. Ballou[1], and J. A. Carroll[2], *[1]Texas Tech University, Lubbock, [2]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, [3]Department of Animal and Food Sciences, Texas Tech University, Lubbock, [4]Department of Agricultural Sciences, West Texas A&M University, Canyon, [5]Cactus Feeders, Canyon, TX, [6]Phileo Lesaffre Animal Care, Milwaukee, WI, [7]Phileo Lesaffre Animal Care, Cedar Rapids, IA.*

Two treatments were evaluated in feedlot heifers to determine the effects of feeding a yeast supplement on metabolic responses to a combined viral-bacterial respiratory disease

challenge. Thirty-two beef heifers (325 ± 19.2 kg) were selected and randomly assigned to one of two treatments: 1) Control (CON), receiving a standard feedlot ration with no yeast supplement, or 2) yeast, (YEAST) control ration plus a combination live yeast (2.5 $g \cdot hd^{-1} \cdot d^{-1}$) and yeast cell wall (2.5 $g \cdot hd^{-1} \cdot d^{-1}$) supplement (Phileo-Lesaffre Animal Care, Milwaukee, WI). Cattle were maintained on treatments for 31 d. On d −3 all cattle were challenged intra-nasally with 1 x 10^8 PFU of bovine herpesvirus-1 (BHV-1) and then allowed to rest in outdoor pens for 3 d. On d 0, each heifer was challenged intra-tracheally with approximately $3x10^7$ CFU of *Mannheimia haemolytica*, was fitted with an indwelling jugular catheter and indwelling vaginal temperature recording device, and was moved into individual stanchions in an environmentally-controlled barn. Whole blood samples were collected at the time of BHV-1 challenge, at 1-h (serum) or 2-h (complete blood cell counts) intervals from 0 to 8 h, and at 12, 24, 36, 48, 60, and 72 h following the *M. haemolytica* challenge. Data were analyzed using the mixed procedure of SAS specific for repeated measures with fixed effects of treatment, time, and their interaction. Cattle in the YEAST group had a greater glucose concentration following *M. haemolytica* challenge (121.38 ± 2.91 vs. 109.86 ± 2.90 mg/dL, respectively; $P < 0.01$) and decreased serum concentrations of blood urea nitrogen compared to CON (11.82 ± 0.53 vs. 10.12 ± 0.53 mg/dL, respectively; $P = 0.03$). There was no difference in serum NEFA concentration between YEAST (0.14 ± 0.01 mg/dL) and CON (0.15 ± 0.01mg/dL; $P = 0.37$). These data indicate that feeding a combination live yeast and yeast cell wall product may modulate energy stores by reducing muscle catabolism to provide energy for the activated immune system in response to a respiratory disease challenge. The reduction in catabolism has the potential to improve live animal performance when an animal is exposed to respiratory diseases.

Key Words: feedlot health, respiratory disease challenge, yeast

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1099 In vivo production, quality and pregnancy of bovine embryos from cows with high or low intake of dry matter or energy. R. Sartori*[1], R. S. Surjus[2], A. B. Prata[2], P. L. J. Monteiro Jr.[1], M. C. C. Mattos[3], F. C. Mattos[4], G. B. Mourao[5], and F. A. P. Santos[6], *[1]University of São Paulo-ESALQ/USP, Piracicaba, Brazil, [2]ESALQ/USP, Piracicaba, Brazil, [3]CEVA Animal Health, Paulinea, Brazil, [4]Ourofino Animal Health, Cravinhos, Brazil, [5]Department of Animal Science, University of São Paulo/ESALQ, Piracicaba, Brazil, [6]University of São Paulo, Piracicaba, Brazil.*

This study evaluated the influence of dry matter (DM) or energy intake on in vivo embryo production and quality. Non-lactating Nelore cows ($n = 32$, 4 to 10 y old) weighing 489.5 ± 11.3 kg and with BCS of 3.3 (1 to 5) were used. After 15 d on a maintenance diet [1.2% of DM per kg of body weight (BW)], cows were randomly divided into four groups. Maintenance (M), 0.7M, and 1.5M received the equivalent of 70, 100, and 150% of the M diet, respectively. The fourth group (Energy; E) received a diet with DM similar to the M group, but with an energy level equivalent to the 1.5M group. Cows were fed individually and offered all diets in a Latin-square arrangement every 42 d. Cows were submitted to a conventional superovulation protocol. Superovulatory response was assessed by ultrasonography and embryo quality was evaluated according to the IETS guidelines, as well as by pregnancy per fixed-time transfer (P/ET) of 274 vitrified embryos to Nelore recipients. Pre-prandial blood plasma insulin was performed by RIA at the onset of superovulation. Data were analyzed by PROC GLIMMIX of SAS ($P < 0.05$). Circulating insulin was greater in the E group (8.7 ± 0.9 μIU/mL) compared with groups 0.7 M (4.6 ± 0.9) and M (5.3 ± 0.85), not differing from group 1.5M (6.6 ± 0.9). Superovulation (CL number) was lower in donors fed high energy (9.7 ± 1.2) compared with cows receiving the standard diet [0.7M (13.0 ± 1.3), M (14.2 ± 1.2) or 1.5M (13.9 ± 1.2)] due to the negative correlation between circulating insulin and CL number ($r = -0.32$). Nevertheless, there was no difference between groups for number of ova/embryos (~6), viable (~3), or freezable embryos (~2.7). Regardless of treatment, circulating insulin was negatively correlated with the number of viable embryos ($r = -0.22$). There was lower P/ET at 60 d in the 97 cows receiving embryos from donors fed high energy (E; 29.4%) compared with the 177 cows receiving embryos from donors fed the standard diet (0.7M, M or 1.5M; 43.3%). Moreover, probability of P/ET at 60 d evaluated by logistic regression decreased as circulating insulin of donor cows increased from 0.64 to 25.0 μIU/mL. In conclusion, there was effect of diet on the superovulatory response and P/ET. Additionally, high circulating insulin of the donors was associated with lower superovulatory response, less viable embryos and less P/ET at 60 d. Financial support from FAPESP and CNPq. We also thank InVitro Brasil and Hildergard Pritzelwitz Experimental Station.

Key Words: cattle, fertility, insulin, nutrition

1100 Body condition score affects milk yield and energy balance of dairy cows after a short or no dry period. A. van Knegsel* and B. Kemp, *Adaptation Physiology Group, Wageningen University, Wageningen, Netherlands.*

Shortening or omitting the dry period of dairy cows is of interest because it limits the negative energy balance in early lactation, mainly due to a reduction in milk yield postpartum. Moreover, there are indications that individual cow characteristics, like parity or genotype, affect the response of dairy cows to a short or no dry period. The objective of this study was to evaluate the effects of prepartum BCS on the response of cows in milk yield, energy balance (EB), and plasma metabolites to a short or no dry period, compared with

a conventional dry period. Holstein-Friesian dairy cows (n = 167) were assigned randomly to three dry period lengths: 0 (no), 30 (short), or 60 d (conventional). Across treatments, cows were classified on prepartum BCS as lean (BCS < 3.0; n = 64), normal (3.0 ≤ BCS < 3.5; n = 60) or fat (BCS ≥ 3.5; n = 43). Feed intake and milk yield were recorded daily from week −8 till 14 relative to calving and averaged per week. Energy balance was calculated weekly. Blood was sampled weekly. Repeated measures ANOVA was performed to analyze the data. Data are expressed as LSMEANS ± SEM. Postcalving, milk production, EB, plasma free-fatty acids, and β-hydroxybutyrate concentration were affected by BCS-class*dry period length interaction (P < 0.05). More specifically, in fat cows milk yield and EB were similar between dry period lengths (milk: 39.9 ± 0.8 kg/d; EB: −113 ± 15 kJ/kg$^{0.75}$*d). In lean cows, however, shortening or omitting the dry period reduced milk yield (short: 38.5 ± 1.0 kg/d; no: 31.1 ± 1.1 kg/d) and improved the EB (short: −56 ± 19 kJ/kg$^{0.75}$*d; no: 89 ± 20 kJ/kg$^{0.75}$*d) compared with a conventional dry period. In normal cows, shortening or omitting the dry period reduced milk yield (short: 38.9 ± 1.2 kg/d; no: 30.1 ± 1.1 kg/d), compared with a conventional dry period. Between lean and normal cows, there were no differences in milk yield reduction or EB improvement due to dry period length. Results for plasma metabolites were in line with results for EB. In conclusion, prepartum BCS affects milk yield and EB of dairy cows after a short or no dry period. This might imply that the optimal dry period length for dairy cows depends on prepartum BCS. Currently, studies are ongoing to develop a decision support tool for dry period length based on individual cow characteristics, like parity and BCS, to optimize milk yield and cow health.

Key Words: energy balance, individual variation, metabolic status

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1101 The effect of stocking rate and cow breed on resumption of cyclicity, blood indicators of energy status, uterine health and reproductive parameters in pasture-based dairy systems.

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Identifying the optimum stocking rate (SR) and cow breed for pasture-based systems is essential to maximize output/ha without compromising reproductive performance. The objective of this study was to compare the performance of two different breeds (Holstein Friesian, HF, and Jersey crossbreds, JEX; n = 69 per breed) on one of three different SR (Low: 2.5 cows/ha; Medium: 2.9 cows/ha; and High: 3.3 cows/ha; n = 46 per treatment). The study was performed over 3 consecutive years. Milk samples were collected three times per week from parturition until week 5 post AI for progesterone analysis. Ten blood samples/cow/year were collected (weeks −2, 1, 2, 3, 4, 6, 8, 10, 14, and 18 relative to parturition) to determine circulating concentrations of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (β-HBA) and insulin-like growth factor-1 (IGF1). Uterine cytology samples were collected on Week 6 after parturition from 252 cows during Years 2 and 3 of the study to determine the proportion of cows with sub-clinical endometritis. The fertility performance of each herd was monitored during a 12-wk breeding season across 3 yr of the study. Days to resumption of cyclicity was not affected by SR or breed (25.2, 24.5, and 25.1 ± 1.2 d for Low, Med, and High SR; 24.7, vs. 25.2 ± 1.7 d for HF vs. JEX, respectively, P > 0.05). Mean plasma glucose (72.9, 71.9, and 72.4 ± 0.9 mg/dl), BHBA (0.61 ± , 0.61, and 0.62 ± 0.01, mmol/l), NEFA (0.46, 0.48, and 0.47 ± 0.01 mmol/l), and IGF1 (93.7, 92.9, and 98.9 ± 4.3, mmol/l) concentrations were not affected by SR (Low, Med, and High, respectively; P > 0.05). Mean glucose concentrations were greater in JEX than HF (73.5 ± 0.7 vs. 71.3 ± 0.8, mg/dl; P < 0.05, respectively), but concentrations of NEFA, β-HBA, and IGF1 were not affected by breed (P > 0.05). Neither SR nor breed affected the proportion of cows with sub-clinical endometritis at Week 6 postpartum (SR: 0.15, 0.14, and 0.27 of cows for low, medium and high SR, respectively; Breed: 0.18 and 0.18 of HF and JEX cows, respectively) or 42d pregnancy rate and final in-calf rate (all P > 0.05). In conclusion, under the conditions of this study, there was no major effect of increased SR or cow breed on reproductive performance. It is important that farm SR allows nutritional requirements to be met and that cows are genetically suited to seasonal-calving pasture based systems.

Key Words: breed, fertility, stocking rate

1102 Implications of acute or chronic pasture restriction on indicators of metabolic status in grass-based dairy cows.

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Annual variation in pasture growth rate has a major effect on grass availability for grazing dairy cows, especially at the onset of lactation in early spring. The objective was to determine the effect of imposing acute (2 wk) or chronic (6 wk) periods of varying levels of pasture restriction on indicators of metabolic health and hepatic gene expression in early lactation dairy cows. Holstein Friesian and Jersey crossbred cows (n = 96) were randomly assigned to one of four daily herbage allowances (DHA: 60, 80, 100, and 120% of intake capacity) for either two or 6 wk (12 cows per treatment) during early

lactation for 2 consecutive years. During the experimental period no supplemental concentrates were fed. Milk samples were collected three times per week for progesterone analysis to determine effects on estrous cyclicity. Blood was collected once weekly during the study to determine circulating concentrations of glucose, non-esterified fatty acids (NEFA), and β-hydroxybutyrate (β-HBA). In Year 2 of the study, liver biopsies were collected from a subset of cows assigned to the 60% DHA for 2 wk, 60% DHA for 6 wk, and the 100% DHA for 6 wk treatments at experimental weeks 0, 2, and 6. Reverse-transcription quantitative PCR (RT-qPCR) analysis was used to determine the mRNA abundance of 24 target genes related to energy metabolism. Data were analyzed using PROC MIXED of SAS. The DHA treatments had no effect on resumption of cyclicity, mean plasma glucose, or NEFA concentrations. Mean plasma β-HBA concentrations during the treatment periods were increased in cows on the restricted DHA treatments for 2 wk (1.25 ± 0.09, 1.08 ± 0.1, 1.03 ± 0.1, 0.9 ± 0.09 mmol/l; $P < 0.05$; 60%, 80%, 100%, and 120%, respectively) and 6 wk (1.43 ± 0.09, 1.32 ± 0.1, 1.06 ± 0.1, 1.04 ± 0.09 mmol/l; $P < 0.05$; 60%, 80%, 100% and 120%, respectively). At week six, 60% DHA increased mean expression of *glucose-6-phosphatase, pyruvate carboxylase, carnitine palmitoyltransferase 1A, acyl-CoA synthetase long-chain 1* and decreased mean expression of *fatty acid synthase and acetyl-CoA-carboxylase.* DHA had no effect on mRNA abundance of *IGF-1*, but 60% DHA for 6 wk increased expression of *insulin-like growth factor binding protein-2*. We conclude that imposing acute periods of restricted DHA had only modest effects on metabolic health in early lactation dairy cows.

Key Words: gene expression, metabolites, restriction

1103 The effects of ketosis, feed restriction, and an endotoxin challenge on circulating serotonin (5-HT) in lactating dairy cows. E. A. Horst*[1], S. K. Kvidera[1], M. Abuajamieh[1], E. J. Mayorga[1], M. A. Al-Qaisi[1], H. B. Green[2], K. M. Schoenberg[2], W. E. Trout[3], and L. H. Baumgard[1], *[1]Iowa State University, Ames, [2]Elanco Animal Health, Indianapolis, IN, [3]Elanco Animal Health, Greenfield, IN.*

Circulating serotonin (5-HT) is thought to be associated with various metabolic disorders and hypocalcemia during the transition period. Objectives were to evaluate the effect of ketosis, feed restriction, or endotoxin challenge (models where energetic and calcium metabolism is markedly altered) in lactating cows on circulating 5-HT. Blood samples were obtained from three separate experiments and circulating BHBA, NEFA, and glucose were measured in all three experiments while ionized calcium was only measured in the endotoxin challenge. Data were analyzed using PROC MIXED and PROC CORR of SAS 9.4. In the ketosis study, blood samples from cows clinically diagnosed with ketosis ($n = 10$) or classified as healthy ($n = 9$) were obtained from a commercial dairy farm at d −7, 3, and 7 relative to calving (Abuajamieh et al., 2015 JDS. 98[2]:876). There was no effect of health status on circulating 5-HT. Circulating 5-HT was negatively correlated with NEFA ($r = -0.47$, $P = 0.04$); however, no other relationships existed between 5-HT and the other metabolites. In the feed restriction experiment (Stoakes et al., 2015 JDS. 98[2]:274), mid-lactation cows were either fed ad libitum ($n = 3$) or restricted to 20% of their ad libitum intake ($n = 5$). There were no effects of feed restriction on circulating 5-HT, and energetic metabolites were not correlated with circulating 5-HT. In a model of endotoxemia (Stoakes et al., 2015 JDS. 98[2]:509), mid-lactation cows were either challenged with lipopolysaccharide (LPS; 1.5 μg/kg BW; $n = 6$) or sterile saline (CON; $n = 6$). LPS decreased blood ionized Ca^{2+} (56%; $P < 0.05$), but had no effect on circulating 5-HT. No relationships existed between circulating 5-HT and energetic metabolites or ionized Ca^{2+}. In summary, ketosis, feed restriction, nor endotoxemia affected circulating 5-HT. Circulating 5-HT was moderately correlated with NEFA in the transition cow experiment, but no other relationships existed between 5-HT and energetic metabolites and calcium in these experimental conditions.

Key Words: feed restriction, ketosis, lipopolysaccharide, serotonin

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1104 Transcriptome analysis reveals fundamental differences between liver of neonatal calves and transition dairy cows. F. Batistel*, M. Vailati Riboni, A. Agrawal, and J. J. Loor, *University of Illinois, Urbana.*

Primary hepatocytes isolated from neonatal calf liver have been used to infer aspects of liver metabolism of dairy cows, with the assumption that physiological responses of neonatal hepatocytes mimic those of cows. To evaluate more directly the usefulness of calf hepatocytes as model to study cow responses, in particular during the early postpartal period, liver RNA from 7 Holstein cows (20 d in milk) and 7 Holstein calves (4-d old) was used for transcriptome analysis. Individual samples were used to determine expression of 7 key metabolic genes via quantitative RT-PCR. Data were $\log_2$ normalized and subjected to ANOVA using the Proc MIXED procedure of SAS. Compared with calves, the expression of genes related with lipoprotein synthesis (*APOB,* $P = 0.01$; *MTTP,* $P = 0.01$), gluconeogenesis (*FBP1*, $P = 0.07$), fatty acid oxidation (*CPT1A,* $P = 0.01$), and methionine metabolism (*MAT1A,* $P = 0.03$; *BHMT,* $P = 0.07$) was lower in calves than cows. Samples were pooled by type (calves or cows) and co-hybrized onto the 44K-Agilent bovine (V2) gene expression microarray chips (Agilent Technologies Inc.). This allowed for a direct comparison of transcriptomes in calves and cows. Out of 14,772 unique annotated genes detected by the array, 354 (2.39%) differentially expressed genes with expression ratio ≥ (calf specific) or ≤ (cow specific) than mean

± 2 SD were considered highly-expressed in calves or cows and used for pathway analysis using the Dynamic Impact Approach (DIA) with the KEGG database. Within this set, 221 (1.49%) had at least 40-fold greater expression in cows (e.g., *ACSL6, APOBEC3A, IGF2BP1, PFKFB3, SLC11A1, VLDLR*) or calves (e.g., *AOX1, CYP1A1, CYP7A1, SLC27A2*). Instead, expression of 73 genes was equal among cows and calves. The 25 most-impacted pathways from the DIA analysis indicated that critical metabolic processes involving amino acid, lipid, carbohydrate, and vitamin metabolism (e.g., branched-chain amino acid degradation; primary bile acid biosynthesis; fatty acid elongation in mitochondria; and glycolysis/gluconeogenesis) were biologically more important and highly-activated in calves than cows. In contrast, signaling pathways related to immunity (e.g., NOD-like receptor, Toll-like receptor, and chemokine), as well as cofactor metabolism (e.g., folate, pantothenate, and CoA biosynthesis) were highly-activated in cows than calves. Overall, results indicate that liver of calves and cows have unique transcriptome profiles, hence, hepatocytes isolated from calves might not be a suitable model to study hepatic function/metabolic responses of cows.

Key Words: calf, dairy cow, liver transcriptome

1105 ADSA®-EAAP speaker exchange presentation: Effect of rumen content exchange on gene expression in rumen epithelium of lactating cows.

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The ruminal epithelium may adapt to changes in diet either by adjusting the absorptive capacity and surface area of papillae or through acute cellular functional adaptations. To test this hypothesis, an experiment involving total rumen content exchange between 3 pairs of lactating cows fed the same diet was performed to investigate the influence of variation in rumen contents and microbial populations therein on gene expression in rumen papillae. Papillae samples were taken during and 1 wk after digesta exchange from the ventral sites of rumen. Sequencing libraries were prepared according to Illumina TruSeq® Stranded mRNA and TruSeq® Small RNA sample preparation. Paired-end sequencing with Illumina HiSeq3000 with 2 × 150 bp read length was used for mRNA (average 50.6 M reads per sample) and single-read sequencing with 1 × 50 bp read length for miRNA (average 2.5 M reads per sample). From mRNA 67.8% of reads were mapped, of which 53.5% were located to known genes, the remaining reads mapping to unannotated regions of the bovine genome. Each animal was analyzed for differential gene expression (DE) between the two time points using edgeR. The *p*-values of DE genes were used for hierarchical clustering to identify groups with different responses. Using this approach experimental animals ($n = 6$) formed two clusters. DE analysis within the clusters revealed 170 genes differentially expressed at FDR < 0.1 in cluster 1 and two genes differentially expressed in cluster 2. The gene set was analyzed with Ingenuity®Pathway analysis (Qiagen). The top five affected canonical pathways were Acyl-CoA hydrolysis, xenobiotic metabolism signaling, dermatan sulfate biosynthesis, chrondroitin sulfate biosynthesis, and IGF-1 signaling. Twelve phenotypes were significantly different between the two clusters before exchange (ANOVA, $p < 0.1$ by permutation test), with cows in cluster 1 having higher milk protein content, lower total tract N digestibility, greater fecal N excretion, and higher molar acetate and lower valerate proportions in rumen VFA. From miRNA libraries 36.3% of reads were uniquely mapped. From 811 annotated miRNAs, 382 miRNAs were expressed. Analyses of miRNA expression between the two clusters revealed miRNAs with large differences before and after the exchange, five of which have been recently reported to be correlated with N efficiency in cows fed low quality forage diets. To conclude, the transfer of digesta contents was associated with alterations in the expression of key genes and gene networks in rumen epithelium.

Key Words: dairy cow, rumen epithelium, transcriptomics

1106 Identification of effects of different forage source on metabolism and function of liver from dairy cows using systematic approaches.

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Liver occupies a unique role in nutritional physiology of lactating dairy cows, but the effects of forage source on its metabolism and function have not been well examined. This study was conducted to investigate the effects of different forage source on liver metabolites and key gene functions in dairy cows using gas chromatography–time of flight/mass spectrometry (GC-TOF/MS) based metabolomics and RNA-seq analysis. A total of 12 multiparous Holstein dairy cows were fed 2 diets with different forage source: Alfalfa hay (AH, $n = 6$) and corn stover (CS, $n = 6$). The multivariate statistical analysis (PCA, PLS-DA, and OPLS-DA) showed a clear separation of metabolomics profiles between AH and CS groups. A total of 270 metabolites were identified in the liver with 28 of them significantly different between the 2 diets (VIP > 1&$P < 0.05$). In AH, 71 up-regulated metabolites ($\log_2$(z-score) > 1 & $P < 0.05$) were associated with gluconeogenesis, vitamin and mineral metabolism, amino acid metabolism, propanoate metabolism, cell death and survival, carbohydrate metabolism, and energy production. In CS, 64 up-regulated metabolites were involved in cellular growth and proliferation, organismal development, cell-to-cell signaling and interaction, molecular transport and lipid metabolism. Three

metabolites, leucine, cystine, and hippuric acid were further identified as biomarkers based on the analysis (AUC value, predicted class probabilities, and predicted accuracy) of different combinations of significantly different metabolites. In addition, expression of 11,781 genes was detected (more than 50% of samples with CPM > 1) in the liver. One gene module containing 122 genes had a significantly strong positive correlation ($R = 0.82$, $P = 0.001$) with cystine abundance by weighted gene co-expression network analysis (WGCNA). The main functions represented by these genes were gluconeogenesis, pyruvate metabolic process, monosaccharide biosynthetic process, carbohydrate homeostasis, glucose homeostasis, hexose biosynthetic process, chemical homeostasis, and vitamin-related metabolic process which were all up-regulated in the AH group. Our results suggest that various metabolites, pathways, and gene functions were significant changed in response to forage source. These can be used for further characterization of the regulatory mechanisms of forage-related milk performance in dairy cows.

Key Words: dairy cow, gene function, liver metabolomics

1107 Early postpartum administration of sodium salicylate to multiparous dairy cattle is associated with alterations in feeding behavior up to 120 d in milk.

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Previous research has indicated that the use of non-steroidal anti-inflammatory drugs such as sodium salicylate following calving can alter milk yield later in lactation. In the current experiment, sodium salicylate was administered following calving, and cattle were observed through 120 d in milk (DIM). Cows in their second parity and greater were blocked by parity and alternately enrolled into 1 of 2 treatments following calving, receiving either drenches of water (CON) or drenches of 125 g of sodium salicylate dissolved in a similar volume of water (SAL) at approximately 24, 48, and 72 h postpartum. A total of 28 animals per treatment were enrolled in this experiment, and 42 of these animals ($n = 21$ cows/treatment) were included in feeding behavior measurements. Of these, 16 cows were in their third parity and greater, and 26 were in their second parity. Feeding behavior was measured by feed bunks suspended from load cells that continuously monitored bunk weight. For all feeding behavior responses, variables (meal weight, meal length, number of meals/d, and intermeal interval) were averaged by day, and daily responses were averaged by week for statistical analysis. No differences were detected due to treatment for milk yield, energy-corrected milk (ECM), or DMI; however, a significant parity by treatment interaction was observed ($P = 0.03$), where SAL decreased intake in second parity cows but not cows in their third parity and greater. This resulted in a tendency for a treatment by parity interaction for milk yield:DMI ($P = 0.08$) and a significant interaction between treatment and parity on ECM:DMI ($P = 0.02$). Similarly, a significant interaction between parity and treatment was observed for average meal weight ($P = 0.04$), with no difference between treatments in second parity animals but increased average meal weight for older cows receiving SAL. Treatment with SAL was associated with fewer daily meals and greater average meal length ($P = 0.03$). A tendency for an interaction between treatment and week was also observed for intermeal interval ($P = 0.06$). For all feeding behavior variables measured, responses to treatment were delayed by at least 5 wk following administration. In conclusion, despite a failure to detect differences due to treatment in milk production or intake, postpartum treatment with sodium salicylate resulted in subtle and prolonged differences in feeding behavior in multiparous cows.

Key Words: feeding behavior, inflammation, sodium salicylate

1108 Proteomic analysis reveals increased abundance of inflammation-related proteins in adipose tissues from postpartum dairy cows treated with sodium salicylate.

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The objective was to investigate the effects of sodium salicylate (SS) on the proteome of adipose tissue in early lactating dairy cows. Holstein cows in parity 3+ were assigned alternately at time of calving to either control or SS treatments. CON treatment received a molasses carrier in drinking water while the SS received 2.5 g/L SS with the molasses carrier in drinking water for 7 d after parturition. Adipose tissue biopsies were obtained from control cows ($n = 5$) and cows treated with SS ($n = 5$) at 7 DIM. Proteins were analyzed by intensity based, label-free quantitative shotgun proteomics at Weizmann Institute of Science (Rehovot, Israel). Proteins were extracted and subjected to in-solution tryptic digestion, followed by nanoflow liquid chromatography coupled to high-resolution tandem mass spectrometry (nanoLC-MS/MS). Quantitative data were extracted using Genedata Expressionist data analysis package and proteins identified using Mascot search engine. PCA analysis revealed two distinctive clusters, therefore proteomics data, after logarithmic transformation, were analyzed by two-way ANOVA for effects of treatment (control vs. SS), cluster (1 vs. 2) and their interaction. Only proteins that were different at $P < 0.05$ for effect of treatment and $P > 0.05$ for effect of interaction, as well as having a fold change (FC) of ± 1.5 were further considered. Proteomic analysis quantified 1422 proteins in adipose tissue, from which the abundance of 80 (5.6%) proteins differed in SS vs. control.

The top canonical pathways affected by SS treatment (IPA, Ingenuity) were the complement system, IL-10 signaling and acute phase response signaling. The abundance of several proteins related to these pathways was altered; for example, complement C1q subcomponent subunit B (C1QB, FC = 360, $P < 0.002$), complement component 1-r subcomponent (C1R, FC = 1.6, $P < 0.04$), flavin reductase (NADPH; BLVRB, FC = 1.6, $P < 0.05$) and lipopolysaccharide-binding protein (LBP, FC = 2.3, $P < 0.004$) were increased in SS adipose compared to controls. These findings imply that SS treatment up-regulates some inflammation-related proteins in adipose tissue, perhaps to maintain the desired inflammatory tone during the subacute inflammation in postpartum cows.

Key Words: adipose, immune, proteomics, sodium salicylate

1109 WS Effect of delayed insemination of non-estrual beef heifers following a 7-d-CO-Synch plus controlled internal drug release (CIDR) insert timed artificial insemination protocol.

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Synchronizing estrus before AI is an effective way to shorten the calving season, and increasing the number of pregnancies per AI may lead to greater use and acceptance of synchronization protocols among beef producers. Our objective was to determine if pregnancy rates to fixed-time AI (FTAI) would be improved by delaying insemination in heifers not expressing estrus before FTAI in a 7-d CO-Synch + controlled internal drug release (CIDR) estrous synchronization protocol. Four hundred sixty-five yearling beef heifers across three locations of commercial and purebred herds were given 100 μg of GnRH (Cystorelin; Merial) i.m. and a CIDR insert (Zoetis; 1.38 g of progesterone) on d 0. On d 7 CIDR inserts were removed and all heifers received 25 mg of $PGF_{2\alpha}$ i.m. (Lutalyse; Zoetis) and were fitted with an estrous detection patch (Estrotect; Rockway, Inc.). Heifers were placed in one of three treatment groups based on estrous detection patch color at 48 h after $PGF_{2\alpha}$: 1. Estrus-Red 48 h (n = 180)—heifers displayed estrus as indicated by red estrous detection patch and were given GnRH (100 μg i.m. Cystorelin) and inseminated at 48 h after $PGF_{2\alpha}$, 2. Non-Estrus-Gray 48 h (n = 137)—heifers did not display estrus by 48 h after $PGF_{2\alpha}$ and were given GnRH (100 μg i.m. Cystorelin) and inseminated at 48 h after $PGF_{2\alpha}$, and 3. Non-Estrus Delayed-Gray 56 h (n = 148)—heifers did not display estrus by 48 h after $PGF_{2\alpha}$ and were given GnRH (100 μg i.m. Cystorelin) at 48 h and inseminated at 56 h after $PGF_{2\alpha}$. Pregnancy data were analyzed using SAS PROC GLIMMIX with treatment as a fixed effect, herd as a random variable, and heifer as the experimental unit. By 48 h after $PGF_{2\alpha}$, 38.7% of all heifers were in estrus (as indicated by a red estrous detection patch). Pregnancy rate to AI was greatest for Estrus-Red 48 h heifers (67.8%; $P < 0.0001$) as compared to heifers in the Non-Estrus-Gray 48 h (39.4%) and Non-Estrus Delayed-Gray 56 h (42.6%) groups. Among heifers not expressing estrus before FTAI, delayed insemination achieved a similar (P = 0.83) percent of pregnancies (Non-Estrus Delayed-Gray 56 h; 42.6%) as compared to Non-Estrus-Gray 48 h heifers (39.4%). Delaying insemination by 8 h in heifers not displaying estrus by 48 h after $PGF_{2\alpha}$ did not improve pregnancy rates to AI.

Key Words: beef heifers

1110 GnRH increased pregnancy risk in suckled beef cows that did not display estrus when subjected to a split-time artificial insemination program.

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We hypothesized GnRH would induce ovulation in a split-time AI program by increasing pregnancy risk (PR) when estrus was not detected. A total of 1236 suckled beef cows at 12 locations in 3 states (CO, KS, and ND) were enrolled. Before applying the fixed-time AI program, BCS was assessed. Cows were treated on d −7 with a CIDR insert concurrent with 100 μg GnRH and on d 0 with 25 mg $PGF_{2\alpha}$ plus removal of the insert. Estrotect patches were affixed to cows at CIDR insert removal. Estrus was defined to have occurred when an estrus-detection patch was > 50% colored (activated). Cows in estrus by 65 h (n = 758; 61.3% of all cows) were allocated randomly to 2 treatments: 1) GnRH and early AI at 65 h (E+G; n = 373), or 2) AI only at 65 h (E–G; n = 385). Remaining cows were allocated randomly to 2 treatments: 1) GnRH injection at 65 h and late AI at 84 h (L+G; n = 252), or 2) AI only at 84 h (L–G; n = 226). Pregnancy was determined 35 d after AI via transrectal ultrasound. Pregnancy risk did not differ (P = 0.68) between E+G and E–G cows (61.9 vs. 60.4%), respectively. Conversely, for cows inseminated at 84 h, PR was greater (P = 0.01) in cows that received GnRH at 65 h compared with their herd mates not receiving GnRH (41.7 vs. 30.8%), respectively. Of those cows not in estrus by 65 h, 57.7% displayed estrus by 84 h for a total expression of estrus by all cows of 77.6%. Pregnancy risk was greater ($P < 0.01$) in cows not detected in estrus by 84 h when treated with GnRH at 65 h compared with no GnRH (+G = 33.4% [n = 146] vs.–G = 15.0% [n = 128]), whereas no difference in PR was detected for cows detected in estrus (+G = 65.3% [n = 103] vs.–G = 61.7% [n = 97]). Neither estrus expression by 65 or 84 h nor pregnancy risk was influenced by BCS, parity, or days postpartum at AI. Cows had greater PR when they displayed estrus before AI and cows

that did not display estrus by 65 h benefited from an injection of GnRH at 65 h before insemination occurred at 84 h.

Key Words: beef cows, GnRH, split-time AI

1111 Comparison of long- versus short-term CIDR-based protocols to synchronize estrus before fixed-time AI in primiparous 2-yr-old beef cows.

J. M. Abel*, B. E. Bishop, J. M. Thomas, M. R. Ellersieck, S. E. Poock, M. F. Smith, and D. J. Patterson, *University of Missouri, Columbia.*

This experiment was designed to compare the 14-d CIDR-PG (14-d) and 7-d CO-Synch + CIDR (7-d) protocols on the basis of estrous response, pregnancy rates resulting from fixed-time AI (FTAI), and final pregnancy rates at the end of the breeding season in primiparous 2-yr-old beef cows. Cows assigned to the 14-d treatment (n = 355) received a CIDR insert on d 0 with removal on d 14. Cows assigned to the 7-d treatment (n = 349) received GnRH and CIDR inserts on d 23. On d 30 CIDRs were removed from cows assigned to the 7-d treatment and $PGF_{2\alpha}$ was administered to cows in both treatments. On d 33 GnRH was administered concurrent with FTAI at 66 and 72 h after $PGF_{2\alpha}$ for 7-d and 14-d treated cows, respectively. Estrus response at FTAI was higher for 7-d compared to 14-d treated cows (7-d = 74%; 14-d = 43%; $P < 0.0001$); however, pregnancy rates resulting from FTAI were similar between treatments (7-d = 64%; 14-d = 63%; $P = 0.52$). Furthermore, 87% and 88% of 14-d and 7-d treated cows conceived within the first 30 d of the breeding season, and final pregnancy rates at the end of the breeding season did not differ between treatments (7-d = 96%; 14-d = 95%; $P = 0.93$). To understand similarities between treatments in pregnancy rates resulting from FTAI, despite differences in estrous response rates before FTAI, ovaries were mapped and serum estradiol-17-β (E_2) concentrations were evaluated among a subset of cows in each treatment. The 14-d treated cows had smaller diameter dominant follicles at $PGF_{2\alpha}$ ($P = 0.04$) and FTAI ($P = 0.002$) compared to 7-d treated cows (10.9 ± 0.3; 13.0 ± 0.3; vs. 11.9 ± 0.4; 14.5 ± 0.3); however, serum E_2 concentrations at $PGF_{2\alpha}$ ($P = 0.06$) and FTAI ($P = 0.001$) were greater for 14-d vs. 7-d treated cows (3.7 ± 0.4; 8.0 ± 0.7 vs. 2.5 ± 0.4; 4.2 ± 0.8). These differences suggest that dominant follicles of 14-d treated cows remain in an active growth stage at the time FTAI is performed compared to 7-d treated cows in which growth of dominant follicles may have plateaued. This theory is supported from previous studies that report decreased aromatase activity in granulosa cells when growth of dominant follicles plateau compared to actively growing follicles. In summary, these data suggest that the 14-d CIDR-PG and 7-d CO-Synch + CIDR protocols may be used to effectively synchronize estrus before FTAI in primiparous 2-yr-old beef cows.

Key Words: artificial insemination, estrus synchronization, primiparous 2-yr-old beef cow

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1112 Comparing split-time AI pregnancy rates among non-estrous heifers based on administration of GnRH at AI.

B. E. Bishop*, J. M. Thomas, J. M. Abel, M. F. Smith, M. R. Ellersieck, S. E. Poock, and D. J. Patterson, *University of Missouri, Columbia.*

This experiment was designed to evaluate split-time artificial insemination (STAI) in beef heifers following administration of the 14-d (d) controlled internal drug release (CIDR)-prostaglandin $F_{2\alpha}$ (PG) protocol and to compare pregnancy rates among non-estrous heifers based on administration of GnRH at AI. Estrus was synchronized for 1138 heifers across six locations. Heifers received a CIDR insert (1.38 g progesterone) on Day 0 with removal on Day 14. Estrus detection aids (Estrotect) were applied at PG (25 mg) 16 d after CIDR removal on Day 30. Treatments were balanced across locations for heifers using reproductive tract score and weight. Split-time AI was performed at 66 and 90 h after PG, and estrus was recorded at these times. Heifers in both treatments that exhibited estrus by 66 h were inseminated at that time and did not receive GnRH, whereas AI was delayed 24 h until 90 h after PG for heifers that failed to exhibit estrus by 66 h. For heifers in treatment 1 that were inseminated at 90 h, GnRH (100 μg) was administered concurrent with AI at 90 h. Heifers in treatment 2 that were inseminated at 90 h did not receive GnRH. Estrous response did not differ between treatments at 66 h after PG (1 = 70%; 2 = 71%; $P = 0.58$) or during the 24 h delay period (1 = 59%; 2 = 52%; $P = 0.21$). There was no effect of treatment on total AI pregnancy rate (1 = 54%; 2 = 56%; $P = 0.60$) or on AI pregnancy rate for heifers inseminated at 66 h (1 = 58%; 2 = 62%; $P = 0.86$) or 90 h (1 = 44%; 2 = 39%; $P = 0.50$) after PG. Ovulation was confirmed via ultrasonography for a subset of heifers that failed to exhibit estrus before 90 h after PG. For heifers that failed to exhibit estrus by 90 h, ovulation rate did not differ between treatments (1 = 52%; 2 = 50%; $P = 0.64$) nor did AI pregnancy rate (1 = 24%; 2 = 15%; $P = 0.97$). In summary, when split-time AI was used in conjunction with the 14-d CIDR-PG protocol in heifers, comparable pregnancy rates were achieved without administering GnRH.

Key Words: beef heifer, gonadotropin-releasing hormone, split-time artificial insemination

1113 Comparing fixed-time artificial insemination to split-time artificial insemination with delayed administration of GnRH in postpartum beef cows. B. E. Bishop*, J. M. Abel, J. M. Thomas, M. F. Smith, S. E. Poock, M. R. Ellersieck, and D. J. Patterson, *University of Missouri, Columbia.*

This experiment was designed to compare pregnancy rates in postpartum beef cows following split-time (STAI) or fixed-time (FTAI) artificial insemination. Estrus was synchronized for 671 cows at seven locations following administration of the 7-d CO-Synch + CIDR protocol (100 μg GnRH + CIDR insert [1.38 g progesterone] on d 0; 25 mg prostaglandin $F_{2\alpha}$ [PG] at CIDR removal on d 7). Cows were assigned to treatments that were balanced across locations based on age, body condition score, and days postpartum at the time treatments were initiated. All cows in treatment 1 (n = 333; FTAI) were inseminated at 66 h after PG and GnRH was administered concurrent with insemination regardless of estrus expression. For cows in treatment 2 (n = 338), STAI was performed at 66 and 90 h after PG, and estrus was recorded at these times. Cows in the STAI treatment that exhibited estrus by 66 h were inseminated at that time and did not receive GnRH, whereas AI was delayed 24 h until 90 h after PG for cows that failed to exhibit estrus by 66 h. Gonadotropin-releasing hormone (100 μg) was administered concurrent with AI at 90 h only to cows failing to exhibit estrus. Estrus expression that occurred during the 24 delay period among cows assigned to the STAI treatment increased the total proportion of cows that expressed estrus before insemination (1 = 60%; 2 = 86%; P < 0.001). Pregnancy rates for cows inseminated at 66 h that exhibited estrus did not differ between treatments (1 = 58%; 2 = 58%; P = 0.93); however, pregnancy rates among non-estrous cows at 66 h was improved (1 = 35%; 2 = 51%; P = 0.01) among cows assigned to the STAI treatment when insemination was postponed by 24 h. Consequently, total AI pregnancy rate tended to be higher for cows that received STAI (1 = 49%; 2 = 56%; P = 0.06). In summary, following administration of the 7-d CO-Synch + CIDR protocol, total estrous response increased and pregnancy rates resulting from AI tended to be higher among cows assigned to STAI versus FTAI treatments.

Key Words: beef cow, fixed-time artificial insemination, split-time artificial insemination

1114 Split-time artificial insemination following synchronization of estrus with the 14-d CIDR-PG protocol in primiparous 2-yr-old beef cows. J. M. Abel*, B. E. Bishop, J. M. Thomas, M. R. Ellersieck, S. E. Poock, M. F. Smith, and D. J. Patterson, *University of Missouri, Columbia.*

This experiment was designed to test the hypothesis that estrous response and pregnancy rate following synchronization of estrus with the 14-d CIDR-PG protocol in primiparous 2-yr-old beef cows would be improved using split-time AI (STAI) compared to fixed-time AI (FTAI). Estrus was synchronized for 523 primiparous, postpartum beef cows at five locations. Cows received a CIDR insert (1.38 g progesterone) on d 0 with removal on d 14. On d 30, 16 d after CIDR removal, cows were administered $PGF_{2\alpha}$ (25 mg) and estrus detection aids (Estrotect) were applied. All cows were administered GnRH (100 μg) on d 33, 72 h after $PGF_{2\alpha}$ administration. Treatments were equally represented across locations, and cows within each location were assigned to one of two treatments based on days postpartum and body condition score. Cows assigned to the FTAI treatment (n = 266) were inseminated at a fixed-time concurrent with GnRH administration at 72 h after $PGF_{2\alpha}$ regardless of estrus expression, while cows assigned to the STAI treatment (n = 257) were inseminated based on estrus expression observed at 72 h. Cows assigned to STAI that expressed estrus by 72 h were inseminated; however, AI was delayed 24 h until 96 h after $PGF_{2\alpha}$ for cows that failed to express estrus before the standard fixed time. Estrus detection aids remained attached following GnRH at 72 h for all non-estrous cows assigned to the STAI treatment, and estrus expression during the delayed time period was recorded. Estrous response at 72 h did not differ between treatments (FTAI = 42%; STAI = 40%; P = 0.33). Delayed insemination to 96 h after $PGF_{2\alpha}$ of STAI treated cows that failed to exhibit estrus before the standard FTAI at 72 h increased total estrous response (FTAI = 42%; STAI = 64%; P < 0.0001); however, pregnancy rates resulting from AI were similar between treatments (FTAI = 56%; STAI = 55%; P = 0.60). In summary, estrus expression was increased when STAI was used following synchronization of estrus with the 14-d CIDR-PG protocol in primiparous 2-yr-old beef cows; however, this strategy did not result in significant improvements in AI pregnancy rates compared to FTAI.

Key Words: artificial insemination, estrus synchronization, primiparous 2-yr-old beef cow

1115 The 9-d CIDR-PG protocol: Incorporation of prostaglandin pretreatment into a long-term, CIDR-based estrus synchronization protocol improves timed AI pregnancy rates in postpartum suckled beef cows. *J.* M. Thomas*, B. E. Bishop, J. M. Abel, J. W. Locke, S. E. Poock, M. F. Smith, and D. J. Patterson, *University of Missouri, Columbia.*

An experiment was designed to test the hypothesis that pregnancy rates after fixed-time artificial insemination (FTAI) among postpartum suckled beef cows would be improved by incorporating pretreatment with prostaglandin $F_{2\alpha}$ (PG) into a long-term, CIDR-based estrus synchronization protocol. The 9-d CIDR-PG protocol, a modified protocol in which PG is used to facilitate a decreased length of progestin treatment, was compared to the 14-d CIDR-PG protocol. Protocols were compared on the basis of initial estrus response following

CIDR removal, final estrus response following the administration of PG, and pregnancy rate resulting from FTAI. Estrus was synchronized for 321 cows across three locations. Treatments were represented across locations, and cows within each location were randomly assigned to one of the two protocols based on age and body condition score (BCS). Cows assigned to the 14-d CIDR-PG treatment received a CIDR insert (1.38 g progesterone) on d 0 with removal of CIDR on d 14; 25 mg PG 16 d after CIDR removal on d 30; and 100 μg GnRH on d 33, 72 h after PG. Cows assigned the 9-d CIDR-PG treatment received 25 mg PG and a CIDR insert (1.38 g progesterone) on d 5; 25 mg PG and removal of CIDR on d 14; 25 mg PG 16 d after CIDR removal on d 30; and 100 μg GnRH on d 33, 72 h after PG. Estrus response following CIDR removal on d 14 did not differ between treatments (87% versus 85%, $P = 0.71$), and there was no difference in final estrus response following the administration of PG on d 30 (53% versus 50%, $P = 0.69$). A significant effect of treatment was found on pregnancy rate resulting from FTAI, with cows assigned to the 9-d CIDR-PG protocol achieving greater FTAI pregnancy rates than cows assigned to the 14-d CIDR-PG protocol (63% versus 53%, $P < 0.05$). Across treatments, greater pregnancy rates ($P = 0.06$) tended to be achieved by cows that expressed estrus before FTAI (69% for 9-d CIDR-PG, 57% for 14-d CIDR-PG) than were achieved by cows that failed to express estrus (57% for 9-d CIDR-PG, 48% for 14-d CIDR-PG). In summary, when using a long-term, CIDR-based estrus synchronization protocol among mature, suckled beef cows, FTAI pregnancy rates are improved through use of the 9-d CIDR-PG protocol.

Key Words: artificial insemination, beef cow, estrus synchronization

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1116 Requirement of GnRH administration at the onset of the 5 d CO-Synch + CIDR protocol in suckled beef cows.

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The objective of this experiment was to evaluate the requirement of GnRH administration at controlled internal drug release (CIDR) insertion in the 5-d CO-Synch + CIDR protocol (5dCO). Postpartum, suckled beef cows ($n = 2159$) from 11 herds at 5 universities were assigned by age, BCS, and days postpartum to receive either: 1) standard 5dCO hormone administration including 100 μL of GnRH at CIDR insert and 2 concurrent 25-mg doses of $PGF_{2\alpha}$ (PG) at CIDR removal (G2PG), 2) no GnRH at CIDR insert and 2 concurrent, 25-mg doses of PG at CIDR removal (NoG2PG), or 3) no GnRH at CIDR insert and a single, 25-mg dose of PG at CIDR removal (NoG1PG). Estrous response between PG and timed-AI (TAI) was determined using estrous detection aids. All cows were TAI 72 h after CIDR removal, concurrent with administration of 100 μL of GnRH. Estrous cyclicity before synchronization was determined using a combination of 2 blood samples collected 10 d apart and estrus detection aids administered approximately 24 d before CIDR insert. Transrectal ultrasonography was used on a subset of cows at both CIDR insert and removal to record all ovarian structures as well as to detect pregnancy 31 to 42 d after TAI. Data were analyzed using the MIXED and GLIMMIX procedures of SAS for continuous and binary response variables, respectively. Herd nested within university was included as a random effect. Number of total follicles and size of the largest 2 follicles at CIDR insertion were not different ($P \geq 0.34$). However, the largest follicle at CIDR removal was greater ($P = 0.02$) in NoG2PG than G2PG and NoG1PG (13.2, 11.5, and 12.1 ± 0.5 mm, respectively). Though estrus response was not different ($P = 0.99$) before TAI, detection aid activation was more advanced ($P = 0.01$) in NoG1PG than G2PG and NoG2PG. However, pregnancy to TAI did not differ ($P = 0.66$) among G2PG (55.4%), NoG2PG (52.8%), and NoG1PG (50.5%) treatments. Cows exhibiting estrus before TAI had greater ($P < 0.001$) TAI pregnancy rates (58.1%) than those not exhibiting estrus (39.3%) and cows determined to be cyclic at synchronization initiation had greater ($P < 0.001$) TAI pregnancy rates (53.6%) than non-cyclic cows (37.1%). In conclusion, TAI pregnancy rates were not negatively affected by removal of initial GnRH in the 5-d CO-Synch + CIDR protocol.

Key Words: 5-d CO-Synch, GnRH, synchronization

1117 Comparison of follicular dynamics and subsequent progesterone profiles in Brahman cows with either two or three ovarian follicular waves.

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The objective of this study was to identify differences in follicular dynamics in a *Bos indicus* bovine estrous cycle with 2 or 3 follicular waves (2FW or 3FW) and the subsequent progesterone profiles. Daily ultrasonography was performed on 15 multiparous Brahman cows through a complete estrous

cycle. Blood samples were collected daily from the coccygeal vein throughout the subsequent estrous cycle to determine serum progesterone concentrations by RIA. The ultrasound images were collected using a SonoSite M-Turbo ultrasound with a 7.5 MHz L52X transducer. Follicular data were analyzed using Proc Mixed procedures and serum progesterone data were analyzed using Proc Mixed procedures specific for repeated measures using SAS v9.3. The first FW from cows with 2 or 3 FW estrous cycles were compared and the ovulatory FW were compared. Size and day (d) of the dominant and largest subordinate follicle did not differ between 2 or 3 FW estrous cycles during either the first or ovulatory FW. As expected the length of the FW differed ($P < 0.01$) during the first (2FW = 11.90 ± 0.73; 3FW = 7.00 ± 1.03) and ovulatory FW (2FW = 10.80 ± 0.90; 3FW = 6.00 ± 1.27) between groups, respectively. The d the largest follicle appeared differed ($P < 0.01$) in the first (2FW = 9.5 ± 0.48; 3FW = 5.6 ± 0.67) and ovulatory FW (2FW = 9.8 ± 1.05; 3FW = 5.6 ± 1.47). The greatest number of antral follicles found differed during the first (2FW = 14.20 ± 1.60; 3FW = 6.00 ± 2.25) ($P < 0.01$) and ovulatory FW (2FW = 21.0 ± 2.00; 3FW = 12.5 ± 2.83) ($P = 0.025$). The serum progesterone profile of the following estrous cycle was normalized by analyzing the first 10 d after estrus (CL growth phase) and the 10 d before the succeeding estrus (CL regression phase). There was a tendency ($P = 0.08$) for an interaction between day and number of FW for the CL regression phase of the estrous cycle. The 3FW cows tended to have greater progesterone concentrations during the last 7d of the regression phase compared with the 2FW. These results suggest that cows with 2FW have greater a number of antral follicles within the first and ovulatory FW and the 3FW cows had increased serum progesterone concentrations during the CL regression phase of the subsequent estrous cycle.

Key Words: Brahman cows, follicular dynamics, serum progesterone.

1118 Effect of a progesterone-based estrous synchronization program for timed AI (TAI) on reproductive performance in a seasonal pasture-based dairy production system.

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The aim of this study was to investigate the effect of progesterone-based TAI programs on fertility in seasonal-calving pasture-based dairy herds. At 10 d before the mating start date (MSD), 840 lactating dairy cows on 3 seasonal-calving farms were blocked based on days in milk (DIM) and parity and randomly allocated to one of three treatments: (i) Control: no treatment, inseminated at detected estrus; (ii) P4-Ovsynch: cows received a 7-d progesterone-releasing intravaginal device (PRID®Delta) with 100 mg of GnRH analog (Ovarelin®) at PRID insertion, 25 mg injection of PGF2α (Enzaprost®) at PRID removal, GnRH at 56 h after device removal and TAI 16 h later; (iii)P4-Ovsynch+eCG: same as P4-Ovsynch, but cows received 500 IU equine chorionic gonadotropin (eCG; Syncrostim®) at PRID removal. At trial initiation all cows that were $\geq$ 30 DIM were ultrasound scanned to assess presence/absence of a corpus luteum (CL) and Body Condition Score (BCS) was also recorded. Pregnancy diagnosis was performed by transrectal ultrasonography 30–35d after insemination. Binary data were analyzed using the GLIMMIX procedure of SAS, and time-dependent data were analyzed using survival analysis. Overall, conception rate was not different between groups (51.0%, 52.0%, and 51.8% for Control, P4-Ovsynch, and P4-Ovsynch+eCG, respectively; $P = 0.9$), but the 21-d pregnancy rate was increased by the synchronization protocols (38.6%, 58.6%, and 53.6%; $P < 0.0001$). Supplementation with eCG at PRID removal did not affect pregnancy rate (53.3 vs. 52.5, P4-Ovsynch vs. P4-Ovsynch+eCG, respectively; $P = 0.9$). Compared to the Control group, synchronization treatments significantly reduced the interval from MSD to conception (36.7, 24.0, and 27.1 d, respectively; $P < 0.001$), and consequently reduced the average days open (87.0, 75.0, and 78.0 d, respectively; $P < 0.001$). Across all treatment groups, DIM at start of synchronization had a significant effect on conception rate (44.3%, 51.1%, and 59.5% for < 60, 60–80, and > 80 DIM, respectively; $P < 0.05$), but parity (49.7%, 51.5%, and 53.9% for parity 1, 2, and $\geq$ 3, respectively; $P = 0.7$), BCS (44.9%, 51.6%, and 58.6% for $\leq$ 2.50, 2.75–3.25, and $\geq$ 3.50, respectively; $P = 0.2$) and presence of a CL (51.7% vs. 51.7%; $P = 0.9$) did not have significant effects on the likelihood of pregnancy per AI. Additionally, there were no two-way interactions detected ($P > 0.05$) between treatment and DIM, parity, BCS, or CL status category. In conclusion, the use of TAI accelerated pregnancy establishment of cows in a pasture-based system by reducing days open, but eCG supplementation at PRID removal did not affect pregnancy rate.

Key Words: eCG, progesterone, synchronization, timed AI

1119 Hepatic gluconeogenic enzymes are differentially altered by methyl-donors choline and methionine in bovine primary hepatocytes. T. L. Chandler*[1], S. J. Bertics[1], B. A. Barton[2], and H. M. White[1], [1]*Department of Dairy Science University of Wisconsin, Madison,* [2]*Balchem Corporation, New Hampton, NY.*

Tricarboxylic acid cycle (TCA) and gluconeogenic carbon flux are controlled by balances of pyruvate carboxylase (*PC*) and phosphoenolpyruvate carboxykinase (*PEPCK*). The lipotropic action of choline and methionine may alter fatty acid (FA) oxidation and gluoneogenic carbon availability. The objective of this experiment was to examine regulation of genes controlling gluconeogenesis in response to increasing concentrations of choline chloride (CC), DL-methionine (DLM), and added FA. Primary hepatocytes isolated from 4 Holstein calves were maintained as monolayer cultures for 24 h in media containing optimal concentrations of essential amino acids and 1.25 m*M* pyruvic acid. Treatments of physiologically relevant concentrations of CC (33, 100, 2000, 4500 μ*M*) and DLM (16, 30, 100, 300 μ*M*), with or without a 1 m*M* FA cocktail, were added to a methionine-free media in a factorial design. After 24 h of treatment, cells were harvested for RNA isolation, cDNA generation, and quantification of gene expression by quantitative PCR. Abundance of mRNA was normalized to the geometric mean of three reference genes. Data were analyzed using PROC MIXED of SAS 9.4 with linear and quadratic contrasts in a model accounting for fixed effect of treatment and random effect of calf and reported as least squares means ± SE. Expression of *PC* tended to be linearly increased ($P = 0.06$) by CC (1.28, 1.42, 1.43, 1.50 ± 0.21 arbitrary units (AU)) and was unaffected ($P > 0.15$) by DLM (1.39, 1.51, 1.38, 1.35 ± 0.21 AU). Although, *mitochondrial PEPCK* (*PEPCKm*) expression was unaffected ($P \geq 0.15$) by CC (1.64, 1.58, 1.60, 1.59 ± 0.5 AU) or DLM (1.49, 1.57, 1.65, 1.69 ± AU), *cytosolic PEPCK* (*PEPCKc*) tended to be linearly increased ($P = 0.11$) by CC (1.03, 1.19, 1.30, 1.57 ± 0.32 AU) and decreased ($P = 0.08$) by DLM (1.60, 1.31, 1.21, 0.97 ± 0.32). Expression of *glucose 6-phosphatase* (*G6P*) was quadratically affected ($P = 0.009$) by CC (1.14, 1.42, 0.99, 1.13 ± 0.30) and unaffected ($P > 0.15$) by DLM (1.14, 1.17, 1.19, 1.17 ± 0.30). Treatment with FA increased ($P < 0.001$) expression of *PC* (1.11 vs. 1.70 ± 0.20 AU), *PEPCKc* (0.55 vs. 2.0 ± 0.27 AU), *PEPCKm* (1.36 vs. 1.84 ± 0.48 AU), and *G6P* (0.93 vs. 1.41 ± 0.29 AU). Coordinated increases in *PC* and *PEPCKc* with increasing CC suggests increased capacity for gluconeogenesis. Conversely, decreased *PEPCKc* without altered *PC* may indicate that DLM may increase TCA capacity but not gluconeogenic capacity. Choline and methionine appear to differentially regulate TCA cycle and gluconeogenesis.

Key Words: gluconeogenesis, methyl-donors, primary hepatocytes

1120 Expression of the putative gonadotropin-inhibitory hormone receptor, NPFFR1, in the anterior pituitary gland of the gilt is affected by age and sexual maturation. C. A. Lents*, J. F. Thorson, and D. J. Nonneman, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Gonadotropin-inhibitory hormone (GnIH) purportedly suppresses secretion of luteinizing hormone (LH) by acting through a G protein coupled receptor (NPFFR1) in the anterior pituitary gland and hypothalamus. The objective of these studies was to determine if expression of mRNA for NPFFR1 in the reproductive neurosecretory axis of gilts differed with age or sexual maturation. In Exp. 1, the anterior pituitary gland was collected from gilts at 24 (weaning), 60, 100, and 140 d of age ($n = 14$ to 16 per age). In Exp. 2, the anterior pituitary gland and medial basal hypothalamus were collected from gilts at 240 d of age. Gilts were classified ($n = 12$ to 14 per classification) as prepubertal, peripubertal, or cyclic (midluteal phase) based on estrus records and ovarian morphology at slaughter. Relative abundance of LHβ, FSHβ, CGA, GnRHR1, and NPFFR1 mRNA in pituitary glands from Exp. 1 and 2 and NPFFR1, GnRH1, NPY, POMC, and RFRP in hypothalami from Exp. 2 was measured with qPCR. Data were analyzed by ANOVA with age or reproductive classification as fixed effects. In Exp. 1, age did not affect expression of GnRHR1 or CGA. Expression of FSHβ at weaning was not different from 60 d of age but was greater ($P < 0.05$) than expression at 100 and 140 d of age. Compared to weaning, expression of LHβ was less ($P < 0.05$) at 60 d of age and greater ($P < 0.05$) at 100 d of age, but not different at 140 d of age. Pituitary expression of NPFFR1 was greater ($P < 0.05$) at 100 d of age compared with all other ages. In Exp. 2, prepubertal gilts had less pituitary expression of LHβ ($P < 0.02$) and greater pituitary expression of FSHβ ($P < 0.04$), CGA ($P < 0.001$), GnRHR1 ($P < 0.01$), and NPFFR1 ($P < 0.001$) than cyclic gilts. Expression of LHβ and GnRHR1 was intermediate in peripubertal gilts. Expression of FSHβ, CGA, and NPFFR1 in the pituitary did not differ between peripubertal and cyclic gilts. Expression of NPFFR1 in the hypothalamus of peripubertal gilts was less ($P < 0.05$) than in cyclic gilts. Reproductive classification did not affect hypothalamic expression of GnRH1, NPY, POMC, or RFRP. Increased expression of NPFFR1 in prepubertal gilts indicates an increased sensitivity to GnIH inhibition of LH secretion. Support: NIFA AFRI 2011–67015. USDA is an equal opportunity provider and employer.

Key Words: gene expression, pig, pubertal development,

1121 Role of focal adhesion molecules in maternal recognition of pregnancy in the mare.
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The mechanism responsible for maternal recognition of pregnancy (MRP) in the mare remains unknown. During early pregnancy the equine conceptus and endometrium communicate to attenuate prostaglandin $F2_{\alpha}$(PGF) secretion thus sparing the corpus luteum and maintaining progesterone production. Based on previous experiments we identified focal adhesion molecules (FAM) as potentially playing a key role in this process. We hypothesize that contact of an embryo with equine endometrium causes (i) a change in FAM transcription and (ii) decrease PGF secretion. We designed an in vitro experiment to test this hypothesis. Endometrial biopsies were obtained from mares in a crossover design, with each mare providing samples from a pregnant and non-pregnant (non-mated) control cycle (n = 3/sample day) on d 9 and 11 post-ovulation, a critical time immediately before and during MRP. Pregnancy was confirmed by ultrasonography and presence of an embryo following uterine lavage. Mares were matched by day and embryos collected were used in co-culture experiment. Endometrial samples were divided and placed in culture with or without contact by an equine embryo for 24 h. Total RNA from endometrial biopsies was evaluated by qPCR using primers designed to detect 22 equine-specific FAM transcripts and ELISA was used to assay PGF content in medium. All comparisons were made within day between groups and pregnancy status. Differential expression of 4 and 6 FAM were noted in samples collected on d 9 ($P \leq 0.02$) and 11 ($P \leq 0.05$), respectively, when compared by pregnancy status alone. No changes were detected in FAM expression in samples collected from pregnant mares due to the presence or absence of an embryo, while 1 and 4 FAM differed when embryos were co-cultured with endometrial samples from non-mated mares at d 9 (P = 0.04) and 11 ($P \leq 0.04$), respectively. Secretion of PGF was not attenuated with embryo contact on d 9 regardless of pregnancy status. Embryo contact resulted in dramatic decreases (P < 0.003) in PGF secretion in samples collected from both pregnant and non-pregnant mares 11 d post-ovulation. These data support our hypothesis that FAM expression is altered with the presence of an embryo and implicates FAM in the modulation of PGF secretion. Together these provide new insight into a potential mechanism for MRP in mares.

Key Words: embryo, endometrium, equine, focal adhesion molecule, maternal recognition of pregnancy

1122 Modification of embryonic resistance to heat shock in cattle by melatonin and genetic variation in HSPA1L.
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Seeking for new methods to reduce the effect of heat stress on fertility we examined 1) whether melatonin blocks inhibition of embryonic development caused by heat shock and 2) whether frequency of a thermoprotective allele for *HSPA1L* is increased in blastocysts formed from heat-shocked zygotes as compared to blastocysts from control zygotes. It was hypothesized that melatonin prevents effects of heat shock on development by reducing accumulation of reactive oxygen species (ROS). Effects of 1 μM melatonin on ROS were determined in Exp. 1 and 2. Zygotes were cultured at 38.5 or 40°C for 3 h in the presence of CellROX® reagent. Culture was in a low [5% (v/v)] oxygen (Exp. 1) or low or high [21% (v/v)] oxygen environment (Exp. 2). Heat shock and high oxygen increased ROS; melatonin decreased ROS. In Exp. 1, for example, fluorescent intensity at 38.5°C was 346 ± 54 and 451 ± 51 and 769 ± 47 and 361 ± 50 at 40°C for control and melatonin-treated embryos (interaction, $P < 0.0001$). Development was assessed in Exp. 3–5. In Exp. 3 and 4, zygotes were cultured in low oxygen + 1 μM melatonin and exposed to 38.5 or 40°C for 12 (Exp. 1) or 24 h (Exp. 2) beginning 8 h after fertilization. Melatonin was not thermoprotective in either experiment. Exp. 5 was performed similarly except that temperature treatments (38.5 or 40°C, 24 h) were performed in a low or high oxygen environment (2 x 2 x 2 factorial design with temperature, melatonin, and oxygen concentration as main effects). Heat shock decreased (P = 0.003) percent of zygotes developing to the blastocyst stage (26 ± 1.5 vs. 20 ± 1.5%) independent of melatonin or oxygen concentration. For Exp. 5, blastocysts were genotyped for a deletion (D) mutation (C®D) in the promoter region of *HSPA1L* associated with thermotolerance. Genotype was affected by temperature (P = 0.002). The percent of blastocysts CC, CD, or DD was 43.3, 28.5, and 28.2% for blastocysts from control zygotes and 32.4, 36.0, and 31.6% for blastocysts from heat-shocked zygotes. It was concluded that 1) lack of effect of melatonin or oxygen concentration on embryonic development means that the negative effects of heat shock on the zygote are not mediated by ROS, 2) previously reported effect of melatonin on fertility of heat-stressed cows might involve actions independent of the antioxidant properties of melatonin, and 3) the deletion mutation in the promoter of *HSPA1L* confers protection to the zygote from heat shock. Perhaps, embryonic survival during heat stress could be improved by selecting for thermotolerant genotypes (Support: BARD US-4719–14).

Key Words: heat shock, melatonin, reactive oxygen species, *HSPA1L*

1123 Transgenerational paternal influence on temperament and growth performance of crossbred beef calves. R. C. Vann[*1], B. P. Littlejohn[2], C. R. Long[3], T. H. Welsh, Jr.[2], and R. D. Randel[4], *[1]MAFES-Brown Loam Experiment Station, Mississippi State University, Raymond, [2]Texas A&M AgriLife Research and Department of Animal Science, College Station, [3]Texas A&M AgriLife Research, Overton, [4]Texas A&M AgriLife Research, Texas A&M University System, Overton.*

The objective was to evaluate the transgenerational paternal influence on temperament characteristics and growth performance in a group of crossbred calves sired by bulls that did or did not experience prenatal stress (PNS). These sires were derived from a purebred Brahman population in which dams were assigned to receive 1 of 2 treatments, control (CTRL; $n = 42$) or PNS ($n = 43$). Cows in the PNS group were subjected to 2 h of transportation at 60, 80, 100, 120, and 140 d of gestation (Littlejohn et al., 2016). From this group, 3 sexually mature control and 3 PNS Brahman bulls were mated with mature cows (20 cows per bull) to produce a second generation of calves. These crossbred calves were evaluated for temperament utilizing pen score (PS; 1 = calm and 5 = excitable), exit velocity (EV; m/sec), and temperament score (TEMP; PS+EV/2) at weaning (d 0; adjusted 205 d), d 28, and d 56. At these same time points body weights were recorded. All data were analyzed using Mixed Models Procedures of SAS. Treatment of sire and sex of calf were included as fixed effects. Male calves had greater birth weights compared to females ($P < 0.001$). Steers had greater adjusted 205 d, d 28, and 56 BW ($P < 0.005$) compared with heifers. Calves from PNS sires had greater ($P < 0.001$) adjusted 205 d BW compared to calves from CTRL sires but this did not carry through d 28 or 56. Male calves from PNS sires had greater ($P < 0.001$) adjusted 205 d BW, male calves from CTRL sires and female calves from PNS sires were intermediate and female calves from CTRL sires had the lowest adjusted 205 d BW. Male and female calves from CTRL sires had the greatest PS ($P < 0.002$), EV ($P < 0.05$) and TEMP ($P < 0.002$) scores at weaning compared to male and female calves from PNS sires. Individual sire influenced ($P < 0.05$) all measures of temperament and BW. Weaning TEMP score was highly ($P < 0.001$) correlated to TEMP scores at d 28 and 56 (0.66 and 0.68, respectively). Calves from CTRL sires had greater TEMP scores at weaning; however, by d 56 these differences had diminished. Calves from PNS sires had greater adjusted 205 d BW; however, these differences in BW at weaning, d 28 or 56 were not apparent where adjustments for age of dam and sex of calf were not included. Temperament measures in PNS were lower than for CTRL calves.

Key Words: beef calves, temperament, transgenerational

1124 DNA methylation is a possible basis of phenotypic alterations observed in suckling Brahman calves. B. P. Littlejohn[*1,2], D. M. Price[1,2], D. A. Neuendorff[2], C. R. Long[2], J. A. Carroll[3], R. C. Vann[4], T. H. Welsh, Jr.[1], and R. D. Randel[2], *[1]Texas A&M AgriLife Research and Department of Animal Science, College Station, [2]Texas A&M AgriLife Research, Texas A&M University System, Overton, [3]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, [4]MAFES-Brown Loam Experiment Station, Mississippi State University, Raymond.*

The objective of this experiment was to examine DNA methylation as a potential basis for phenotypic alterations observed in prenatally stressed (PNS) compared to control calves (Littlejohn et al., 2016). Previously, 41 of 85 mature Brahman cows were transported for 2-h periods at 60, 80, 100, 120, and 140 d of gestation, while the remaining cows were controls ($n = 44$). All calves born to control and transported dams (PNS group) were evaluated to determine phenotypic differences in temperament, circulating concentrations of cortisol, immune

Table 1124.

Table 1. Enumeration of strongly hypermethylated and strongly hypomethylated genes in PNS (n=7) compared to control (n=7) bull calves.

	Immune Function	Metabolic Function	Behavior/ Stress/Neural Function	Reproductive Function	Cell Signaling/Gene Function
Strongly Hypermethylated Genes	4	13	3	2	25
Strongly Hypomethylated Genes	14	15	9	2	23

function, and metabolism as suckling calves. At 28 d of age each calf was restrained for collection of jugular blood samples. Buffy coat cells were harvested from whole blood and stored at −80 C. DNA was isolated from buffy coat cells of 7 PNS and 7 control bulls using phenol-chloroform extraction procedures and the samples were analyzed using reduced representation bisulfite sequencing (Zymo Research; Irvine, CA) to determine differential methylation of DNA. Reported genes were differentially methylated ($P < 0.015$) in PNS compared to control calves (Table 1). Genes that were defined as strongly hypermethylated ($n = 41$) were $\geq$ 33% more methylated in PNS than control bulls, while genes that were defined as strongly hypomethylated ($n = 49$) were $\geq$ 33% less methylated than controls. Reported genes were related to immune function, metabolic function, behavior/stress/neural function, reproductive function, and cell signaling/gene function. Several genes were ascribed to multiple functions. Differentially methylated genes related to phenotypic alterations observed in PNS compared to control bull calves suggest epigenetic programming of biological systems in utero.

Key Words: calves, DNA methylation, prenatal stress

1125 Photoperiod manipulations during the dry period significantly impact mammary circadian clock in goats. S. J. Mabjeesh[*1], A. Shamay[2], K. Plaut[3], C. Sabastian[1], and T. M. Casey[3], *[1]Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University, Rehovot, Israel, [2]Institute of Animal Science, The Volcani Center, Bet Dagan, Israel, [3]Department of Animal Sciences, Purdue University, West Lafayette, IN.*

Exposing goats to short day photoperiod (SDPP; 8 h light:16 h dark) during the dry period increases milk production compared to long day photoperiod (LDPP; 16 h light:8 h dark) exposure, due in part to increased mammary cell proliferation rates. Photoperiod information is sent to the master clock in the suprachiasmatic nuclei (SCN) via the retinal nerve. In turn the SCN sends temporal information out to peripheral clocks located in every tissue of the body to synchronize physiological systems to time of day and season. Studies support mammary clock regulates cell proliferation, thus we hypothesized photoperiod effects on milk production are mediated in part by changes in the molecular clock located in mammary gland. The objective of this study was to determine the effect of photoperiod manipulation during the dry period in goats on core clock gene expression in mammary gland. Multiparous Israeli Saanen goats ($n = 6$) were blocked at dry off (?45 d prepartum) into 2 treatments: LDPP ($n = 3$) and SDPP ($n = 3$) based on body weight and previous milk production. All goats were housed in metabolism chambers equipped with two separate but identical environmentally controlled rooms in which photoperiod was adjusted according to the treatment. Goats were fed a total mixed ration in two equal meals at 0800 and 1500 to meet nutritional demands. Serial mammary biopsies were taken over a 24 h period from each goat during 3 wk prepartum at 4 h intervals (0900, 1300, 1700, 2100, 0100, 0500). Tissue was placed in Trizol and immersed in liquid nitrogen. Total RNA was isolated and q-PCR was used to measure expression of two reference genes (BACTIN and GAPDH) and the core clock genes CLOCK and ARNTL. Relative gene expression was calculated using delta-delta CT method with mean of SDPP treatment as normalizer. Exposure to SDPP significantly increased ARNTL ($P < 0.05$) while significantly decreasing CLOCK gene expression. CLOCK and ARNTL heterodimerize to function as a transcription factor, thus changes in their abundance due to photoperiod manipulation will affect expression of target genes, including those that regulate cell proliferation.

Key Words: ARNTL, CLOCK, photoperiod

1126 Management and genetic components of fertility indicators in dairy cattle. T. M. Goncalves[*1], D. Gonzalez-Pena[2], H. Jeong[1], P. J. Pinedo[3], J. E. P. Santos[4], G. M. Schuenemann[5], G. J. M. Rosa[6], R. O. Gilbert[7], R. C. Bicalho[8], R. Chebel[4], K. N. Galvão[9], C. M. Seabury[10], W. W. Thatcher[11], and S. L. Rodriguez Zas[12], *[1]University of Illinois at Urbana-Champaign, Urbana, [2]Zoetis, Kalamazoo, MI, [3]Colorado State University, Fort Collins, [4]University of Florida, Gainesville, [5]Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, [6]University of Wisconsin-Madison, Madison, [7]Cornell University College of Veterinary Medicine, Department of Clinical Sciences, Ithaca, NY, [8]Cornell University, Ithaca, NY, [9]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, [10]Texas A&M University, College Station, [11]Department of Animal Sciences, University of Florida, Gainesville, [12]University of Illinois, Urbana.*

Management and genetic strategies are employed to attain high fertility rates in dairy farms. These high rates in turn enable higher profits from higher milk production, higher replacement rates, higher genetic progress, and lower expenses compared to those in systems with lower fertility efficiency. The goal of this study was to characterize the joint effect of management and genetic variation on fertility indicators that are linked to the cost-effectiveness of diary production systems. Fertility, disease, production, environment, and pedigree records from over 6000 Holstein cows across the U.S Pacific, Southeast, Midwest, and Southwest regions were analyzed. Binary fertility variables included pregnancy at first and second artificial insemination (AI), pregnancy loss after first and second AI, ovarian cyclicity status, and open status +100 d after calving. Explanatory variables included AI method, farm, lactation number, season, body condition

score at 35 d post-calving, milk yield during the first three test-days, retained placenta, twin calving, dystocia, ovarian cyclicity status, open status +100 d after calving, pregnancy at first AI, and pregnancy loss after first AI. Sire of the cow was the random effect in the models. First lactation cows had significantly higher odds of pregnancy at first AI, higher odds of +100 d open status, lower odds of cyclicity than later lactation cows. The odds of pregnancy loss after first and second AI tended to be lower in first lactation cows relative to higher lactation cows. Cyclicity was significant and negatively associated with the odds of pregnancy loss at second AI and was positively correlated with pregnancy loss at first AI, albeit not significant. Calving of twins significantly reduced the odds of cyclicity. Retained placenta and timed AI were associated with significantly higher odds of +100 d open status than estrus-guided AI and not retained placenta, respectively. Higher body condition score was positively and significantly associated with odds of cyclicity. The odds of pregnancy after first and second AI were lower among cows calving during summer relative to winter; likewise, the odds of pregnancy loss after first and second AI were higher during the summer. Heritability estimates for the fertility variables studied ranged from 0.03 (pregnancy at first AI) to 0.12 (pregnancy loss at first AI). These results highlight availability of genetic variation and the major relevance of non-genetic component on fertility indicators. These findings contribute to a long-term multistate project database (USDA-NIFA-AFRI-003542) for direct measures of fertility.

Key Words: milk, pregnancy, reproduction

1127 Effects of OmniGen-AF® on superovulation response and embryo quality in donor beef cows.

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Superovulation is a required yet costly and biologically stressful procedure in cattle embryo transfer. High variation in the number of ova recovered, fertilization rates, and embryo quality result in inconsistent results and prevent full optimization of the procedure for genetic improvement. Inflammation and immune system dysregulation have been suggested to be one cause of this variability. Therefore, the objective of this study was to evaluate OmniGen-AF® (OG) supplementation on superovulatory response, embryo quality, and serum cortisol in beef cattle embryo donors using two doses of follicle stimulating hormone (FSH). Twenty-four cross-bred beef cows were split into four groups and superovulated with 200 or 400 mg FSH and fed OG at 0 or 56 g/hd/day. The feeding period was 49 d. The superovulation protocol was started on Day 28 of feeding and ova were nonsurgically recovered 7 d after estrus and artificial insemination. Good to excellent quality morulae and blastocysts were either fixed for staining or cultured to evaluate in vitro embryo development and plasminogen activator (PA) production. In cows superovulated with 400 mg FSH, feeding OG decreased the percent degenerate embryos recovered ($p = 0.08$). Embryos recovered from cows superovulated with 400 mg FSH and fed OG produced more total PA, with a trend for peak PA production to be higher at 72 h of culture ($p = 0.08$), compared to all other groups. In addition, serum cortisol concentration was significantly lower ($p = 0.049$) in donor cows fed OG at the last breeding of the superovulation protocol compared to controls. In summary, feeding OmniGen-AF may ameliorate negative effects of the higher FSH dose used in superovulation protocols resulting in more transferable and fewer degenerate embryos. Also based on PA production, there is a potential for healthier embryos, with a greater likelihood of developing beyond hatching in an embryo transfer procedure.

Key Words: embryo, OmniGen-AF®, superovulation

1128 OmniGen-AF® reduces basal plasma cortisol as well as cortisol release to adrencocorticotropic hormone or corticotrophin releasing hormone and vasopressin in lactating dairy cows under thermoneutral or acute heat stress conditions.

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Differences in the adrenal cortisol response of OmniGen-AF® (OG) supplemented and control dairy cows to a corticotrophin releasing hormone (CRH) and vasopressin (VP) or an adrenocorticotropic hormone (ACTH) challenge when housed at different temperature-humidity indices (THI) were studied. Holstein cows ($n = 12$; 162 ± 1 DIM) were balanced for milk yield, BW and DIM and randomly assigned to 1 of 2 trts: 1) OminGen-AF, supplemented with OG at 56 g/hd/day for 70 d; or 2) Control (CON), no supplement. Cows were moved to individual tie stalls in 1 of 2 temperature controlled chambers on d 45 and fitted with indwelling rectal temperature (RT) devices and jugular catheters on d 52. Initially THI was cycling at thermoneutrality (TN; THI < 72 for 24 h/d) for 10d, followed by heat stress (HS, THI > 72 for 12h/d) for 10 d. Cows were challenged with CRH (0.3 μg/kg BW) and VP (1 μg/kg BW) at 1000 h on d 6 of TN (d 53 of study) and d 1 of HS (d 57 of study), and with ACTH (0.1 IU/kg BW) at 1000 h on d 7 of TN and d 2 of HS. Blood samples were collected from −2 to 8 h at 30-min intervals relative to each challenge and serum was analyzed for cortisol and corticoid-binding globulin (CBG). Mean

serum cortisol concentration before challenge was lower in OG fed cows compared to CON (9.24 vs. 15.80 ng/ml, $P < 0.003$). Mean serum cortisol concentration was also lower in OG-fed cows compared to CON challenged with ACTH during both TN (27.2 vs. 43.4 ng/ml, $P < 0.01$) and acute HS (11.2 vs. 47.8 ng/ml, $P < 0.01$). Mean plasma cortisol concentrations tended to be lower in OG-fed animals compared to CON cows infused with CRH-VP during TN (38.2 vs. 44.9 ng/ml, $P < 0.06$) and were lower than CON cows infused with CRH-VP during acute HS (49.8 ng/ml vs. 78.3 ng/ml, $P < 0.01$). Mean serum CBG concentration was lower following ACTH infusion than following CRH-VP (753.2 vs. 913.3 ng/ml, $P < 0.01$). OG supplementation had no effect on serum CBG concentrations under TN or HS conditions in this study. However, serum CBG concentrations were elevated by HS in both CON and OG-fed animals following CRH-VP infusion, (1033 vs. 795 ng/ml, $P < 0.01$). Basal serum cortisol was reduced in cows supplemented with OG. In addition, the cortisol response to ACTH and CRH-VP was reduced in OG-fed cows compared to CON and this difference was enhanced during acute heat stress.

Key Words: ACTH, cortisol, heat stress, OmniGen-AF

1129 Reproductive performance with automated activity monitoring or a timed insemination program for first insemination in dairy cows.

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The objective of this study was to compare reproductive performance in lactating cows inseminated exclusively with timed artificial insemination (TAI) or with maximal use of an automated activity monitoring (AAM) system for the first insemination postpartum. From April 2014 to December 2015, a total of 998 cows in two herds in Ontario were randomly assigned to be inseminated at 85 ± 3 d in milk (DIM) following a Double Ovsynch protocol (DO), or be inseminated following detection of estrus by the AAM system between 50 and 75 DIM. In the AAM group, if estrus had not been signaled by 75 DIM, cows received the Ovsynch protocol and were inseminated at 85 ± 3 DIM. After first insemination, cows were managed according to routine herd management programs (combination of AAM and timed AI). The odds of pregnancy at first insemination and by 88 DIM were used as the outcome for logistic regression models. Models were adjusted for herd and parity as fixed effects, and interactions between treatment and covariates were tested. Analyses were done on cows that completed the protocol as assigned (completed protocol basis, $n = 719$) and on all cows that were not culled before first insemination (intention to treat basis, $n = 849$). The odds of being pregnant to first insemination were higher for cows in the DO group than in the AAM group in the intention-to-treat analysis (0.56 vs. 0.42, $P = 0.05$), but were not statistically significant (0.58 vs. 0.45, $P = 0.12$) for the completed protocol analysis. The odds of being pregnant by 88 DIM tended to be higher for cows in the AAM group than in the DO group, but was not statistically different for the completed protocol (0.74 vs. 0.59, $P = 0.13$) or the intention–to-treat analyses (0.70 vs. 0.56, $P = 0.11$).There was an interaction of treatment with herd in both models, such that more cows in the AAM group were pregnant by 88 DIM in one herd, but there was no difference in the other. In this study, the exclusive use of Double Ovsynch had a higher probability of pregnancy at first AI than AAM, but earlier insemination in the AAM group and the possibility of re-insemination resulted in no statistical difference in the proportion of cows pregnant by 88 DIM. There were differences in the relative performance of TAI and AAM between herds.

Key Words: automated activity monitoring, Double Ovsynch, reproductive performance

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1130 Establishing fertility benchmarks for in-line automated milk progesterone monitoring in postpartum dairy cows.

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Milk progesterone (MP4) concentrations in postpartum dairy cows are indicative of ovarian cyclicity. The adoption of automated in-line MP4 monitoring on farm has created the need for benchmarks to better understand MP4 data across cows, parities, farms, etc. The objective was to establish useful fertility benchmarks for in-line MP4 records. MP4 records ($n = 135,588$) from an automated milk progesterone sampling system (Herd Navigator; DeLaval International, Tumba, Sweden) were used. The records were from 1224 lactations of 1505 lactating cows in 4 European herds from January 2014 to December 2015. Farms started monitoring MP4 by 20 d postpartum. Excluded cows did not have MP4 samples before 30 d postpartum, lacked a defined ovulation (MP4 < 3ng/mL followed consecutively by MP4 > 3ng/mL), or lacked consistent MP4 records. The LIFETEST and GLM procedures of SAS 9.4 (Cary, NC) were used to test for differences among farm, parity, and milk yield for commencement of luteal activity (CLA) and length of postpartum estrous cycle. CLA was defined by MP4 > 3ng/mL on 20 to 22 d for initial samples or by a defined ovulation thereafter. Length of estrous cycle was the difference between the two ovulations. Cows were classified as primiparous, mature (2 to 4 lactations), or aged (> 4 lactations). The UNIVARIATE procedure was used to classify daily milk yield (Q1: < 25 kg/day, Q2: 25 to 33 kg/day, Q3: 34 to 41kg/day, Q4: > 41kg/day). The mean interval to CLA was 28.7 ± 14.6 d for all farms. Farm 4 had more cows (103/308 cows) not achieving CLA by 50 d postpartum than the other farms ($P < 0.001$). Aged cows (28/114, 25%) failed to achieve CLA by 50 d postpartum compared with primiparous cows (13/148; $P < 0.05$). Highest producing cows (Q4) failed to

achieve CLA by 40 d postpartum compared with cows producing less than 33kg/day ($P < 0.001$). Length of estrous cycle was shorter for farms 1 and 2 (21.8 ± 0.4d) compared with 22.7 ± 0.4 and 23.0 ± 0.4d for farms 3 and 4, respectively ($P < 0.05$). Length of estrous cycle differed ($P < 0.001$) for primiparous, mature, and aged cows, 21.3 ± 0.2d, 22.7 ± 0.2d, and 23.0 ± 0.5d, respectively. Strategic sampling of MP4 concentrations using an automated system identified differences between herds, parities, and milk production with respect to ovarian cyclicity. Establishing benchmarks based on these data will enable producers to assess ovarian function and the underlying causes of infertility in their herds.

Key Words: fertility, in-line milk progesterone, ovulation

1131 The effects of aspirin on pregnancy rates and pregnancy specific protein B in lactating dairy cows during the summer. J. A. Spencer*[1], K. G. Carnahan[1], B. Shafii[1], J. Dalton[2], and A. Ahmadzadeh[1], [1]*University of Idaho, Moscow,* [2]*University of Idaho, Caldwell.*

The occurrence of embryonic loss in cattle may be due to a hormonal imbalance and untimely secretion of $PGF_{2\alpha}$ around the time of maternal recognition on Days 14–16 after fertilization. The objective of this study was to examine the effect of aspirin, a non-steroidal anti-inflammatory drug, on pregnancy rates (PR) and blood pregnancy specific protein B (PSPB) in lactating dairy cows bred more than once and during the summer months. On Day 14 after two or more AI, 556 cows, from a commercial drylot herd in the Pacific Northwest, were assigned randomly to aspirin (total of 187.2 g; $n = 277$) or control ($n = 279$) treatment groups. Aspirin was administered orally with a balling gun 24 h apart on Day 14 and 15 (93.6 g/dose) after AI, whereas the control group was subjected to oral stimulation. On Day 25 following AI, blood samples were collected from a subset of cows ($n = 192$) and measured for PSPB concentrations. Pregancy status was determined by palpation per rectum between Day 32 to 40 post-AI. The maximum daily ambient temperature ranged from 27 to 39.4°C during the trial period. To estimate the effect of aspirin on PR/AI, a logistic regression model was used. Concentrations of PSPB were analyzed using the analysis of variance. There were no differences in PR/AI ($P > 0.05$) between aspirin (21.7%) and control (27.6%) groups. There was no effect of parity (primiparious 26.2% vs. multiparous 23.8%) or number of inseminations (TBRD) (second and third 26.2% vs. ≥ 4 21.5%) on PR/AI. There was also no effects of treatment or treatment by pregnancy status on PSPB concentrations ($P > 0.05$). Blood PSPB concentrations were 122.8 ± 8.1 and 127.8 ± 6.4 pg/mL for the aspirin and control groups, respectively. However, PSPB concentrations tended to be greater in multiparous cows compared with primiparous cows (132.7 ± 5.7 vs. 118.1 ± 7.3 pg/mL, $P = 0.07$). In addition, PSPB concentrations tended to be greater ($P = 0.07$) for second and 3rd TBRD (133.1 ± 5.3 pg/mL) than ≥ 4 TBRD (117.6 ± 7.8 pg/mL). These results indicate that aspirin may not have an effect on PR/AI or PSPB concentrations in lactating dairy cows subjected to two or more AI during hot summer months in the Pacific Northwest.

Key Words: aspirin, dairy cow, fertility

1132 Temporarily decreasing progesterone after timed artificial insemination decreased expression of ISG15 in blood leukocytes, serum PSPB concentrations, and embryo size in lactating Holstein cows. P. D. Carvalho, C. E. Consentini, S. R. Weaver, R. V. Barletta, L. L. Hernandez, and P. M. Fricke*, *Department of Dairy Science, University of Wisconsin, Madison.*

Our objective was to evaluate the effect of temporarily decreasing progesterone (P4) after timed artificial insemination (TAI) in dairy cows. Lactating Holstein cows ($n = 80$) were synchronized for first TAI using a Double-Ovsynch protocol, and were randomly assigned to receive 12.5 mg $PGF_{2\alpha}$ (dinoprost tromethamine) 5 d after the last GnRH treatment (LowP4) or serve as untreated controls (HighP4). Blood samples were collected thrice weekly from 5 to 32 d after TAI for all cows and from 32 to 67 d for pregnant cows, and were analyzed for P4 and PSPB concentrations. Expression of interferon-tau stimulated gene 15 (ISG15) was assessed in blood leukocytes 18 and 20 d after TAI. Pregnancy diagnosis was performed weekly using ultrasound from 32 to 67 d after TAI, and embryo size (crown-rump length) was measured 32, 39, and 46 d after TAI. Data were analyzed by ANOVA and logistic regression using the MIXED and GLIMMIX procedures of SAS. LowP4 cows had less ($P < 0.01$) P4 than HighP4 cows from 6 to 11 d after TAI, however, pregnancy outcomes 32 d after TAI [43% (17/40) for both treatments, $P = 0.97$] and pregnancy loss from 32 to 67 d after TAI [6% (1/17) vs. 6% (1/17) for LowP4 and HighP4, $P = 0.84$] did not differ between treatments. HighP4 cows diagnosed pregnant 32 d after TAI had greater ($P < 0.05$) expression of ISG15 20 d after TAI than LowP4 cows diagnosed pregnant 32 d after TAI (7.7 vs. 4.9-fold increase from d 4). Pregnant HighP4 cows had greater ($P < 0.01$) PSPB concentrations from 25 to 67 d after TAI than pregnant LowP4 cows. Embryo crown-rump length did not differ between treatments 32 and 39 d after TAI, but HighP4 cows had larger ($P < 0.05$) embryos 46 d after TAI (26.0 ± 1.0 vs. 23.5 ± 1.0 mm). We conclude that treatment with 12.5 mg of $PGF_{2\alpha}$ 5 d after induction of ovulation temporarily decreased P4 concentrations from 6 to 11 d after TAI without inducing luteal regression. Decreasing P4 after TAI decreased expression of ISG15 in blood leukocytes 20 d after TAI, serum PSPB concentrations 25 to 67 d after TAI, and embryo size 46 d after TAI but did not affect P/AI in lactating Holstein cows. Supported by USDA NIFA Hatch project 1006519.

Key Words: embryo, ISG15, progesterone, PSPB

1133 Effects for fertility of processing steps of a new technology platform for producing sexed sperm. M. A. Faust*, J. Betthauser, A. Storch, and S. Crego, *ABS Global, Inc., De Forest, WI.*

To understand the importance for fertility of key processing steps used in the production of sexed sperm, we conducted a series of field trials in U.S. dairy herds. Steps studied were: staining and processing in the absence of laser excitation (1), excitation laser power (2), and the presence of bisected sperm and debris resulting from laser-based cell destruction (3). For experiment 1, split or coincident collections from 8 dairy bulls were used to produce STAINED-L (2×10^6 motile sperm/straw) and untreated controls (CON-H, 10×10^6 sperm). The STAINED-L comprised sperm stained using Hoechst 33342 and subjected to all steps of the sexing process but with no exposure to laser excitation. Conception rates (CR) in Holstein heifers were 56.1% ± 2.8% (n = 312 pregnancy diagnoses) for STAINED-L and 66.7% ± 2.6% (n = 330) for CON-H. For experiment 2, one collection from each of 4 Holstein bulls was split to create untreated CON-L and stained treatments receiving low, medium, and high laser power during excitation. Post-thaw, these 4 treatments contained similar numbers of progressively motile sperm/straw (0.90 to 1.41 $\times 10^6$). When used in virgin heifers, CR for all laser excited treatments were lower than CON-L ($P < 0.01$), and were 58.7% ± 2.0% (n = 591) for CON-L and 45.2% ± 3.6% (n = 186), 44.9% ± 3.5% (n = 205), and 39.1% ± 3.5% (n = 192) for low, medium, and high laser excitation power, respectively. For experiment 3, TRT-L containing 3×10^6 bisected sperm + 2×10^6 unprocessed sperm was compared to matched untreated controls (CON-L, 2×10^6 sperm), and contemporaneously produced high dosage controls (CON-H, 10×10^6 sperm). The TRT-L was produced by processing an aliquot from each ejaculate through the laser detection + laser-destruction system set to bisect 90% of sperm and after each 1 h of collection, combining equal portions of bisected and unprocessed spermatozoa. Post-thaw, matched low dosage fractions contained similar numbers of progressively motile sperm/straw (1.1 to 1.7 $\times 10^6$ for TRT-L and CON-L), but differed markedly in percentage of bisected sperm (69 to 79% and 32 to 59% non-motile, respectively). For CR in virgin heifers, TRT-L did not differ from CON-L but was lower than CON-H ($P < 0.01$). Conception rates were 43.5% ± 1.9% (n = 666) for TRT-L, 43.7% ± 1.9% (n = 668) for CON-L, and 58.1% ± 1.4% (n = 1334) for CON-H. Staining of sperm, excitation laser power, and sperm dosage have implications for the fertility of sexed sperm produced using this novel technology. Contrary to theory, the presence of significant numbers of non-motile and bisected spermatozoa and their debris did not impact conception rates.

Key Words: conception rate, flow cytometer, sperm sexing

1134 Fertility and sex of calf results from a new commercial scale technology platform for producing sexed sperm. M. A. Faust*, J. Betthauser, S. Crego, and A. Storch, *ABS Global, Inc., De Forest, WI.*

By 2016, sexed sperm constitutes ~8% of AI breedings in dairy and beef cattle. Further growth in the application of sexed sperm is limited by the existing technology. We developed a novel technology for producing sexed sperm at commercial scale to better address current and future needs of genetics companies and their producer customers. For this new technology platform, purpose-built microfluidics and laser based cell destruction components were invented to enable accurate quantification of sperm DNA content and rapid and efficient destruction of unwanted cells while safeguarding sperm of the desired chromosomal content. To assess performance resulting from the sexed sperm, field trials were conducted in dairy herds across the U.S. Experiments compared sexed sperm (SEXED, 2×10^6 motile sperm per straw) and non-sexed controls (CON, 10×10^6 sperm) produced using split or contemporaneous collections from a total of 26 bulls (Holstein and Jersey). All treatments were packaged and cryopreserved in 0.25 mL straws. Quality checks for the new sexed sperm product were developed and included an estimate of numbers of progressively motile sperm per straw and a fluorescence in situ hybridization assay for determining sex chromosome content of live sperm. For experiment 1, post-thaw numbers of progressively motile sperm/straw ranged from 0.75 to 1.5 $\times 10^6$ for SEXED batches, 3.9 to 7.7 $\times 10^6$ for CON-Citrate, and 4.1 to 8.9 $\times 10^6$ for CON-Tris. Conception rates from virgin dairy heifers were 61.4% ± 1.5% (n = 1091 pregnancy diagnoses) for citrate control, 60.7% ± 1.5% (n = 1079) for CON-Tris, and 37.4% ± 1.5% (n = 1082) for SEXED. For experiments 2 and 3, no Tris control was used. Numbers of progressively motile sperm/straw ranged from 4.2 to 8.7 $\times 10^6$ for CON and from 0.74 to 1.6 $\times 10^6$ for SEXED batches. In experiment 2, conception rates in dairy heifers were 65.2% ± 1.5% (n = 1005 pregnancy diagnoses) for CON and 46.2% ± 1.6% (n = 1025) for SEXED. Conception rates for experiment 3 were equally favorable and were 64.1% ± 1.6% (n = 900) for CON and 48.3% ± 1.7% (n = 895) for SEXED. Sex chromosome content for live sperm was 87.2% X-bearing (weighted average) and ranged from 73% to 93% for individual batches of SEXED; as indicated by others, proportion of female calves as reported on-farm was somewhat lower at 84.6% ± 1.3% (n = 775, experiments pooled) for SEXED. Proportion of female calves for CON was 50.5% ± 1.4% (n = 1187, pooled). Our novel sexed sperm technology delivers a new platform enabling preselecting the sex ratio of offspring.

Key Words: conception rate, sexed sperm, sex ratio

1135 A meta-analysis of the impacts of maternal weight and fetal sex on uterine blood flow and maternal heart rate in beef cows from mid- to late-gestation. A. R. Tanner*[1], M. L. Bauer[1], V. C. Kennedy[1], B. Mordhorst[1], L. E. Camacho[2], K. C. Swanson[1], and K. A. Vonnahme[1], [1]*North Dakota State University, Fargo,* [2]*University of Arizona, Tucson.*

Uterine blood flow plays a critical role in the development of the conceptus, allowing for the maternal-fetal exchange of nutrients, hormones, and wastes. The objective was to examine the relationships between maternal BW, fetal sex, uterine blood flow, and maternal heart rate in beef cows during mid- to late-gestation. A total of 4 studies were used in the analyses which included 108 beef cows with blood flow measurements taken via Doppler ultrasonography on 4 d of gestation which resulted in 333 total observations. Fetal sex, BW class (45-kg increments), and body weight class by fetal sex interactions were analyzed with generalized least squares using the mixed procedure of SAS with repeated measures. Day of gestation was included as a covariate and treatment was nested within study. Linear, quadratic, and cubic orthogonal contrasts were tested. Cows carrying bull calves ($n = 82$) had greater ($P = 0.03$) uterine blood flow from d 100 to 250 of gestation compared to cows carrying heifer calves ($n = 26$; 18.46 ± 0.764 vs. 15.56 ± 1.04 L/min). As maternal BW increased, uterine blood flow tended ($P = 0.09$) to increase linearly (14.5 mL per kg). Maternal heart rate also increased linearly ($P = 0.02$) as maternal BW increased (0.03 beats per min per kg). Fetal sex did not impact maternal heart rate ($P = 0.13$). In conclusion, the increase in uterine blood flow for male progeny may be contributing to heavier birth weights when compared to their female counterparts. Also, increasing maternal weight may be associated with increased uterine blood flow and heart rate. Perhaps the reason bull calves are heavier than heifer calves at birth may be due to the male's ability to increase uterine blood flow.

Key Words: fetal sex, maternal body weight, uterine blood flow

1136 Validation of a chemical pregnancy test in dairy cows that uses whole blood, shortened incubation times, and visual readout. L. M. Mayo*[1], S. G. Moore[1], S. E. Poock[1], W. Silvia[2], and M. C. Lucy[1], [1]*University of Missouri, Columbia,* [2]*University of Kentucky, Deceased, Lexington.*

Chemical pregnancy testing is an alternative to traditional methods of pregnancy diagnosis in postpartum dairy cows. The objective was to validate a new chemical pregnancy test that confers the advantages of using whole blood (EDTA), plasma (EDTA), or serum, rapid incubation times, and visual readouts. Blood and milk samples were collected from Holstein cows ($n = 320$) 162 ± 62 d (Mean ± SD) postpartum on a confinement farm in Northeast Missouri at 28 d after timed artificial insemination (TAI). Cows were assayed for pregnancy-associated glycoproteins (PAG) by using a new rapid visual ELISA assay, and plasma and milk-based ELISA assays (IDEXX, Westbrook, ME). Transrectal ultrasonography (TU) diagnosis for pregnancy at 35 d or 38 d after TAI was the reference standard for all PAG tests. The optical density (OD) measured with a microtiter plate reader (plasma, milk, and rapid tests) or visual readout (rapid test) were used to diagnose pregnancy. When the OD was used, the percentage of pregnant cows ($n = 159$; TU diagnosed pregnant) classified correctly (sensitivity) for the plasma, milk, and rapid tests were 97 ± 1%, 96 ± 2%, and 95 ± 1% (± SE), respectively. The sensitivity of the rapid test when assessed visually was 98 ± 1%. The specificity (proportion of non-pregnant cows classified correctly) for the plasma, milk, and rapid was 94 ± 2%, 94 ± 2% and 93 ± 2%, respectively. The lesser specificity for visual readouts (85 ± 3%) was associated with faint visual signals that yielded false positive diagnoses. Primiparous cows had greater (0.35 ± 0.02) rapid OD than multiparous cows (0.30 ± 0.01; $P = 0.03$). First insemination cows had a greater signal than cows with multiple breedings at time of sampling for rapid, milk, and plasma PAG assay OD ($P < 0.001$). In a second experiment, lactating Holstein cows ($n = 291$) from 4 Kentucky commercial confinement dairy farms were tested for pregnancy 25 to 95 d after artificial insemination with the rapid visual test. The OD of the rapid visual test followed the known profile for PAG in circulation (high concentrations during early pregnancy followed by a period of lesser concentrations with increasing concentrations thereafter; $P < 0.05$). Overall, the new rapid visual test has equal sensitivity and accuracy to existing PAG tests.

Key Words: pregnancy-associated glycoprotein, pregnancy diagnosis, whole blood

1137 Effects of parity and mid-gestation nutrient restriction on umbilical blood flow, fetal and placental measurements, and birth weight in sheep. M. A. Vasquez*, K. C. Swanson, and K. A. Vonnahme, *North Dakota State University, Fargo.*

We recently reported that mid-gestation (d 50 to 90) nutrient-restriction decreases umbilical blood flow (UBF) and placentome area (PA), and increases pulsatility index (PI) and resistance index (RI) on Day 80 of gestation in multiparous Dorset ewes. The same nutritional restriction applied in nulliparous ewe lambs decreased UBF by d 70 (Lemley et al. (2012).AJP.302:R454). We hypothesized that multiparous ewes would be more resilient to restriction compared to nulliparous ewes. On d 50 of gestation, adult (15 mo) nulliparous (NUL; $n = 12$) and multiparous (MUL; $n = 16$) Dorset ewes carrying singletons were randomly assigned to receive 100% of NRC recommendations (CON) or 60% of CON (RES). On d 91, RES ewes were realimented to 100% of NRC

recommendations. On d 50, and every 10 d until d 110, fetal and placental measurements and umbilical hemodynamics were obtained via ultrasonography. Lamb birth weights were recorded. The study was conducted as a 2 by 2 factorial arrangement of treatments with repeated measures. Data were analyzed using the MIXED procedure of SAS. By d 60 RES ewes were lighter than CON ewes ($P < 0.01$), and remained lighter throughout the experiment. There were no three way interactions or main effects of treatments on UBF, PI, RI and PA ($P \geq 0.57$). There was a parity by day interaction ($P < 0.05$) for RI, but UBF was not affected by parity or diet. At birth no differences were observed in lamb weight ($P \geq 0.78$). Restriction from d 50 to 90 does not appear to impact umbilical hemodynamics or conceptus growth in adults, regardless of parity. Our laboratory's previous observation that reduced UBF in young ewes (6 mo old) resulting from nutrient restriction may be due to maternal age. Future studies investigating age, parity, and body fat reserves of the dam on umbilical hemodynamics are underway.

Key Words: pregnancy, realimentation, sheep

1138 Comparing two ultrasound devices to determine antral follicle counts in dairy cows.

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The determination of antral follicle count (AFC) in dairy cows and its association with fertility is an area of interest to many researchers. Different types of ultrasound scanning (US) devices are available in the market but their comparative efficacy in determining AFC is rarely reported. In this study, we compared the efficacy of EASI-SCAN (BCF Technology Ltd., Rochester, MN; 4.5–8.5 MHz linear array transrectal transducer) and ALOKA (Aloka Co Ltd., Tokyo, Japan; 7.5 MHz linear array transrectal transducer) in the determination of AFC in dairy cows. Eighteen lactating Holstein cows were randomly subjected to transrectal ultrasonography by EASI-SCAN and ALOKA scanners approximately 30 min apart. All scans were performed by the same individual and AFC of each ovary was recorded on separate sheets by a second individual who was blinded to the study. The MEANS procedure of SAS was applied to obtain the mean and standard error of mean for AFC on the left ovary, right ovary and in total. The AFC in left and right ovaries, and total AFC obtained by EASI-SCAN and ALOKA were compared using CORR procedure of SAS and differences determined by TTEST procedure of SAS. The mean (± SEM) AFC determined by EASI-SCAN and ALOKA for the left ovary, right ovary, and total AFC were 6.3 ± 0.7 vs. 9.5 ± 1.0, 7.2 ± 1.4 vs. 11.0 ± 1.8, and 13.6 ± 2.0 vs. 20.5 ± 2.7, respectively. Likewise, the range for the total AFC was lower with EASI-SCAN (4 to 35) than with ALOKA (7 to 46). Although AFC data obtained by EASI-SCAN and ALOKA were significantly correlated ($r = 0.90$; $P < 0.0001$), total AFC was approximately 34% lower ($P < 0.01$) with EASI-SCAN than with ALOKA. Findings indicated that the ALOKA (7.5 MHz transducer) is more precise in determination of AFC than EASI-SCAN (4.5–8.5 MHz transducer).

Key Words: antral follicle count, dairy cows, ultrasound scanner

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1139 The repeatability of antral follicle count and anti-Müllerian hormone concentration at two different postpartum stages in dairy cattle.

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Low antral follicle count (AFC) and low plasma anti-Müllerian hormone (AMH) concentrations are reportedly associated with poor reproductive outcomes in dairy cattle. The primary objective of this study was to evaluate the repeatability of AFC and plasma AMH concentrations at two different postpartum stages. The ovaries of 100 lactating Holstein cows were subjected to transrectal ultrasonography (Aloka Co Ltd., Tokyo, Japan) by a single individual using a 7.5 MHz linear array transducer to determine AFC at 14 ± 1 and 75 ± 1 d postpartum (second exam was approximately 48 h after the second GnRH of an Ovsynch protocol). Blood samples were also collected 14 ± 1 and 75 ± 1 d postpartum to determine plasma AMH concentrations. The MEANS procedure of SAS was used to obtain the mean and standard error of means for the continuous variables, and correlations between continuous variables were tested using the CORR procedure of SAS. The means (± SEM) for AFC determined at 14 and 75 d postpartum were 27 ± 1 and 23 ± 1, respectively. Likewise, the mean (± SEM) AMH concentrations determined at 14 and 75 d postpartum were 190.0 ± 12.8 and 224.8 ± 14.3 pg/mL, respectively. A moderate significant correlation ($r = 0.41$; $P < 0.01$) existed between AFC at 14 and 75 d postpartum. Concentrations of AMH at 14 and 75 d postpartum were strongly correlated ($r = 0.75$; $P < 0.01$). The correlation between AFC and plasma AMH was moderate at both 14 ($r = 0.57$; $P < 0.01$) and 75 d ($r = 0.59$; $P < 0.01$) postpartum. Results indicate that both AFC and AMH concentrations are repeatable at different stages postpartum, although the repeatability is significantly greater for AMH.

Key Words: anti-Müllerian hormone, antral follicle count, repeatability

1140 Dairy cows with shorter ano-genital distance may be more fertile than those with longer ano-genital distance. M. Gobikrushanth*[1], T. C. Bruinjé[1], M. G. Colazo[2], and D. J. Ambrose[1,2], [1]*Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, Canada,* [2]*Livestock Research Branch, Alberta Agriculture and Forestry, Edmonton, Canada.*

The ano-genital distance (AGD) is the distance from anus to base of the penis in male, or clitoris in female mammals. The AGD is a reflection of testosterone exposure during in utero development. Thus, AGD in the adult is indicative of prenatal androgen exposure and reportedly associated with several reproductive health outcomes in humans and laboratory animals. The objectives of this preliminary study were: (1) to characterize variations in the measurements of AGD in a population of dairy cows and (2) to determine associations between categories of AGD and traditional reproductive indices. The AGD in 93 lactating Holstein cows (35 primiparous and 58 multiparous) were measured using a digital caliper. To increase precision, the average of three AGD measurements in each animal was used. All cows were subjected to fixed-timed AI at ~75 d postpartum following a Presynch/Ovsynch protocol. The traditional reproductive indices of first service conception rate, number of inseminations and days open were determined for the current lactation. Cows were ranked based on AGD, from shortest to longest, and those in the top and bottom 50th percentiles were classified into SHORT (n = 46) and LONG (n = 47) AGD categories. The continuous variables were analyzed using the MIXED procedure of SAS and first service conception rate was modeled against categories of AGD and parity as interactions between categories of AGD and parity were not significant, and analyzed using LOGISTIC procedure of SAS. The overall AGD ranged from 95.7 to 149.0 mm and mean (± SEM) AGD were 111.3 ± 0.9 and 129.2 ± 1.1 mm for cows categorized as SHORT and LONG AGD groups, respectively. Cows in the SHORT AGD group tended (P = 0.07) to have 2.4 times higher odds of becoming pregnant to the first insemination than cows in LONG AGD. Similarly, cows in the SHORT AGD group tended to have fewer days open (142.5 ± 10.4 vs. 169.5 ± 11.6 d; P = 0.10) and required less number of inseminations (2.3 ± 0.2 vs. 2.8 ± 0.2; P = 0.12) than those in LONG AGD. The AGD ranged from 95.7 to 142.7 mm (mean ± SEM; 119.6 ± 1.2 mm) for primiparous cows and from 100.0 to 149.0 mm (mean ± SEM; 120.8 ± 0.9 mm) for multiparous cows. The first service conception rate, number of inseminations and days open did not differ between primiparous and multiparous cows. In summary, findings of this preliminary study suggest that cows with shorter ano-genital distance tend to have better reproductive performance than cows with longer ano-genital distance.

Key Words: ano-genital distance, dairy cows, fertility

1141 Pregnancy Associated Glycoprotein (PAG) concentrations in early gestation from dairy heifers undergoing embryo transfer. S. Reese[1], M. H. Pereira[2], J. L. M. Vasconcelos[3], and K. G. Pohler*[1], [1]*University of Tennessee, Knoxville,* [2]*UNESP-FMVZ, Botucatu, Brazil,* [3]*Sao Paulo State University, Botucatu, Brazil.*

Diagnosing and identifying successful pregnancies early in gestation has important economic and management implications for dairy producers. The limitation of current ultrasound and chemical based pregnancy diagnosis methods are that they are most effective starting at Day 30 of gestation. Pregnancy associated glycoproteins (PAGs) are produced by the ruminant placenta and can be used to accurately detect pregnancy as early as Day 28 of gestation. More recent data indicate that circulating PAGs may also be a marker of embryonic viability and predictive of embryonic mortality after Day 28 of gestation. The objective of the current study was to determine if early gestation circulating PAG levels could be indicative of pregnancy for an individual heifer following a baseline sample. Our hypothesis was that Day 24 PAG levels could be predictive of pregnancy if there was a significant increase over a Day 17 baseline sample. In vitro produced embryos were transferred into synchronized virgin percentage Holstein dairy heifers (n = 206) using timed embryo transfer (TET). Blood was collected at Day 17 and 24 for PAG analysis as well as Day 31 for confirmation of pregnancy. Serum concentrations of PAG were quantified using an in house PAG ELISA with antibodies raised against PAGs expressed early in gestation (Green et al., 2005, Pohler et al., 2013). Following TET there were 101 heifers identified pregnant on Day 31 of gestation (49%) using ultrasound and PAG testing. Circulating concentrations of PAG were significantly different ($P < 0.05$) at d 24 of gestation between heifers that were pregnant (2.98 ng/mL) and non pregnant (0.69 ng/mL). In addition, when using receiver operating curve (ROC) analysis PAGs were 95% accurate in diagnosing pregnancy at Day 24 of gestation when circulating PAG levels reached 1.39 ng/mL (~50% of pregnant heifers). When determining pregnancy only based on subtracting the Day 17 sample (baseline) from Day 24, we were able to accurately diagnosis 79% of the heifers for pregnancy at d 31 of gestation. Interestingly, about 11% of heifers following TET that ended up being not pregnant at Day 31 of gestation had increased Day 24 circulating PAGs compared to the Day 17 baseline sample. Thus suggesting that pregnancy loss occurred between Day 24 and 31 of gestation in these heifers. In summary, early pregnancy detection may be possible by using PAGs; however more work is needed to refine this area.

Key Words: heifers, PAGs, pregnancy

1142 Protein kinase A directly phosphorylates GSK3β, and regulates β-catenin via phosphorylation in granulosa cells. B. H. Aloqaily*[1], C. A. Gifford[2], B. I. Gomez[1], and J. A. Hernandez Gifford[1], *[1]Oklahoma State University, Stillwater, [2]Department of Animal Science, Oklahoma State University, Stillwater.*

Estradiol serves an important role in female fertility and FSH drives estradiol production. Beta-catenin is a transcriptional co-factor that is required for FSH-induced estradiol production. Beta-catenin is activated via phosphorylation at Ser^{552} and Ser^{675} by protein kinase A (PKA) dependent event. Wingless-type mouse mammary tumor virus (WNT) also regulates the β-catenin pathway. Glycogen Synthase Kinase-3 β (GSK3β) is a component of the β-catenin degradation complex; canonical WNT signaling pathway phosphorylates protein kinase B (AKT) and inhibits GSK3β resulting in β-catenin accumulation in the cytoplasm. Work in our laboratory demonstrated that WNT downregulates steroidogenesis. Furthermore, AKT is required for β-catenin accumulation and FSH-induced estradiol production, suggesting convergence of the WNT and FSH pathways. We hypothesize that WNT inhibition of FSH signaling occurs through modulation of phosphorylation patterns on β-catenin. The objective of these experiments was to evaluate the phosphorylation pattern of β-catenin in response to PKA, AKT, and WNT signaling pathways. Granulosa cells (KGN cell line) were cultured and treated with vehicle control, phosphoinositide 3-kinase (PI3K) inhibitor LY294002 (LY) (30 μM; 30 min), Forskolin (FSK) (10 μM; 1.5 h), WNT (50 ng/mL, 30 min) and the combination of these treatments. Western blot was used to detect total and phosphorylated β-catenin and phosphorylated GSK3β. Protein abundances were analyzed using densitometry software and densitometry values for treatments were analyzed using the GLM procedure of SAS. When significant model interactions were detected, means were separated using PDIFF. Treatment of FSK combined with WNT and LY enhanced GSK3β phosphorylation compared to control ($P < 0.05$). Similarly, FSK alone or in combination with WNT and LY enhanced ($P < 0.05$) phosphorylation of β-catenin at Ser^{675} and Ser^{552}, but WNT did not alter β-catenin phosphorylation after FSK stimulation ($P > 0.10$). However, when the AKT pathway was blocked with LY, FSK, and WNT treatment increased phosphorylation of GSK3β and subsequently β-catenin at Ser^{552} suggesting that PKA can directly phosphorylate GSK3β. Results from these experiments demonstrate that WNT does not alter FSH-stimulated phosphorylation patterns of β-catenin indicating that WNT inhibits FSH signaling via other unknown mechanisms.

Key Words: beta-catenin, FSH, WNT

1143 Plasma anti-Müllerian hormone in dairy heifers and associations with reproductive performance in two reproductive programs for first artificial insemination. T. V. Silva[1], J. E. P. Santos[2], and E. S. Ribeiro*[3], *[1]Department of Animal Sciences, University of Florida, Gainesville, [2]University of Florida, Gainesville, [3]Department of Animal Biosciences, University of Guelph, Guelph, Canada.*

The objective was to investigate associations between plasma anti-Müllerian hormone (AMH) and reproductive performance in dairy heifers inseminated after detection of estrus or synchronized ovulation. Heifers ($n = 601$) in three farms were enrolled in the study 6 d before moving to the breeding pens. A randomized complete block design was used to assign one of two reproductive programs (RP) for first artificial insemination (AI): estrous detection (ED, $n = 297$) or timed AI (TAI, $n = 304$). Heifers in the ED group had their tailheads painted with chalk and were evaluated for signs of estrus once daily. Heifers receiving TAI were enrolled in the 5-d TAI protocol. On the day of enrollment, blood was sampled and analyzed for concentrations of AMH using an immune assay (AnshLite Bovine AMH CLIA). Within farm, concentrations of AMH in plasma were ranked and categorized as low (20% lowest values), high (20% highest values), or intermediate (60% intermediate values). Reproductive performance was evaluated for 84 d and the remaining AIs were performed after detection of estrus in both RP groups. Pregnancy was diagnosed on Days 32 and 60 after AI. Binary data were analyzed by logistic regression using the GLIMMIX procedure of SAS, and pregnancy rate was analyzed by the Cox's proportional hazard model using the PHREG procedure of SAS. Statistical models included the effects of AMH, RP, and their interaction, farm and semen. Concentration of AMH in plasma ranged from 2.6 to 566.3 pg/mL, and averaged 12.9, 30.9, and 85.4 pg/mL for low, intermediate and high AMH, respectively. Pregnancy per AI on Days 32 and 60 after first AI were not affected by AMH (d 32: 57.5, 65.9, and 64.2%, $P = 0.21$; d 60: 56.7, 62.6, and 58.3%, $P = 0.38$; for low, intermediate, and high AMH, respectively) or RP (d 32: 62.3, and 65.5%, $P = 0.16$; d 60: 58.2, and 62.8%, $P = 0.11$; for ED and TAI, respectively). Compared with low AMH, rate of pregnancy during the 84 d was similar for intermediate (AHR = 1.19, $P = 0.12$) and high AMH (AHR = 1.11, $P = 0.46$). Nonetheless, TAI had a faster rate of pregnancy (AHR = 1.58; $P < 0.01$) than ED. No interactions between AMH and RP were detected. In conclusion, plasma AMH was not associated with reproductive performance of dairy heifers in neither one of the two RP evaluated.

Key Words: AMH, heifers, timed AI

1144 Wingless-type mouse mammary tumor virus integration site (WNT) regulation of ovarian thec cells of cattle. L. J. Spicer*, *Oklahoma State University, Stillwater.*

During ovarian follicular development, granulosa and theca cell (TC) proliferation and differentiation are influenced by gonadotropins, insulin-like growth factors (IGF), and diverse intraovarian factors. Based on high-density microarray analysis comparing cystic and noncystic bovine follicles, we discovered that secreted frizzled related protein 4 (SFRP4) mRNA is downregulated in granulosa cells of cystic follicles suggesting that the WNT system may be involved in cyst formation. Numerous WNT ligands bind to several cognate Frizzled receptors (FZD), and SFRP4 is a truncated form of FZD capable of blocking the action of WNT ligands. Dickkopf-1 (DKK1) is another WNT antagonist and R-spondin-1 (RSPO1) one of a group of four secreted proteins that enhance Wnt/β-catenin signaling. Our overall hypothesis is that granulosa cells signal TC via SFRP4, DKK1, RSPO1, and WNT secretion to regulate TC differentiation and proliferation during follicular development. Therefore, in vitro experiments were conducted to study the effects of WNT family member 3A (WNT3A), RSPO1, DKK1, IGF1, and fibroblast growth factor-9 (FGF9) on bovine TC proliferation and steroidogenesis. TC of large (8 to 20 mm) follicles were collected from ovaries of beef cattle (n = 20 per replicate) and cultured for 48 h and then treated in serum-free medium for 48 h containing either no additions (control), 30 or 100 ng/mL of recombinant human WNT3A, RSPO1, DKK1, IGF1 and/or ovine LH. At least 7 follicles from 5 or more cattle were used to generate one biological replicate per experiment and this was repeated thrice. Each treatment within a biological replicate was replicated 2 or 3 times. For each set of replicated experiments, ANOVA was conducted using SAS. In experiment 1 using LH-treated TC, both IGF1 and WNT3A increased ($P < 0.05$) cell numbers and androstenedione production, whereas WNT3A inhibited ($P < 0.05$) progesterone production by 30%. In experiment 2 using IGF1 plus LH-treated TC, WNT3A (30 ng/mL) further increased ($P < 0.05$) IGF1-induced androstenedione production from 770 to 930 ± 60 pg/10^5 cells/24 h. Similarly, in experiment 3, 100 ng/mL of RSPO1 further increased ($P < 0.05$) IGF1-induced androstenedione production. In experiment 4, SFRP4 and DKK1 alone had no significant effect on TC proliferation or steroidogenesis. In experiment 5, FGF9 blocked ($P < 0.05$) the WNT3A-induced increase in androstenedione production. We conclude that the ovarian WNT system is functional in cattle, increasing proliferation and androstenedione production of TC.

Key Words: cattle, theca cells, WNT3A

1145 Plasma concentrations of glucagon-like peptide 1 and 2 in calves fed calf starters containing lactose. Y. Inabu*[1], A. Saegusa[2], K. Inouchi[2], M. Oba[3], and T. Sugino[1], *[1]Hiroshima University, Higashi-hiroshima, Japan, [2]ZEN-RAKU-REN, Nishi-shirakawa, Japan, [3]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada.*

The objective of this study was to evaluate the effect of lactose inclusion in calf starters on plasma concentrations of glucagon-like peptide 1 (GLP-1) and 2 (GLP-2). Holstein bull calves (n = 60) were raised on an intensified nursing program using milk replacer containing 28.0% CP and 15.0% fat, and fed the texturized calf starter containing lactose at either 0 (Control), 5.0 (LAC5), or 10.0% (LAC10; n = 20 for each treatment) on a DM basis. All calf starters were formulated for 23.1% CP. Ethanol soluble carbohydrate concentration of Control, LAC5, and LAC10 starters were 7.3, 12.3, and 16.8%, respectively. Starch concentrations of Control, LAC5, and LAC10 were 29.7, 27.0, and 21.4%, respectively. All calves were fed treatment calf starters ad libitum. Blood samples were obtained weekly from 1 wk to 11 wk of age and used to measure plasma GLP-1, GLP-2, insulin, and β-hydroxyl butyric acid (BHB) concentrations. Plasma BHB concentrations were higher ($P < 0.01$) for LAC10 (169 ± 5.1 μmol/L; LSM ± SEM) compared with Control (153 ± 4.8 μmol/L) and LAC5 (148 ± 5.2 μmol/L). Plasma GLP-1 and GLP-2 concentrations were not affected by treatments. However, relative values of plasma GLP-1 concentrations compared with that of the baseline (1 wk of age) were higher ($P < 0.01$) for LAC10 (94.8 ± 5.01%) compared with LAC5 (66.5 ± 5.11%), and for LAC5 compared with Control (42.5 ± 4.73%), and similar tendency was observed for GLP-2 concentrations relative to that of the baseline (80.6 ± 5.42%, 74.7 ± 5.43%, and 73.3 ± 5.3%, respectively, for LAC10, LAC5, and Control, respectively; P = 0.09). Plasma insulin concentrations were lower ($P < 0.01$) for LAC5 (4.69 ± 0.58 ng/mL) and LAC10 (4.60 ± 0.58 ng/mL) compared with Control (5.52 ± 0.58 ng/mL). Lactose intake was positively correlated with plasma BHB concentrations (Spearman's correlation coefficient; $r_s = 0.87$, $P < 0.01$), and tended ($r_s = 0.41$, $P = 0.07$) to be positively correlated to plasma GLP-1 concentrations, but not correlated with plasma GLP-2 concentrations. In addition, plasma GLP-1 concentrations were positively correlated with plasma concentrations of BHB ($r_s = 0.85$, $P < 0.01$). In conclusion, these results indicate that inclusion of lactose in calf starters may contribute to maintaining high plasma concentrations of GLP-1, which was associated with greater plasma BHB concentrations.

Key Words: calf, glucagon-like peptide, lactose

1146 Metabolic profile and inflammatory response in calves with different intake of immunoglobulins.

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To achieve a successful passive transfer of immunoglobulins in newborn calves, it is recommended to feed the colostrum quickly after birth, paying attention to the quantity and quality of colostrum administered. The quality of colostrum is associated with content of Immunoglobulins G (IgG), which can vary dramatically among cows. The conventional strategy calls for feeding a calf of 45 kg first colostrum with at least 100 g of IgG. The adequate intake should be monitored measuring the concentration of IgG in the colostrum or in the blood of calves after 24–48 h from the first meal. Moreover, besides IgG for which determination is complex, other blood parameters might provide information on amount of IgG intake and eventual consequences on health. The aim was to assess the relationship among IgG intake with average daily gain (ADG), metabolic profile, inflammatory response and oxidative stress using 45 Holstein calves over the first month of life. After colostrum analyses, calves were retrospectively divided in two groups: G1 (n = 24), ingesting less than 100 g of IgG (average intake = 68 g) from first colostrum, and G2 (n = 21), ingesting more than 100 g of IgG (average intake = 133 g), from first colostrum (1.5 L of colostrum). Besides frequent blood samples, daily health status and body weight also were recorded. Our results showed a poor correlation between density and IgG content of colostrum (r = 0.34), while the correlation between blood IgG and gGT was high (r = 0.9 P < 0.001). During the first 4 wk of life, G1 compared with G2 calves had more clinical problems and lower ADG (0.27 vs. 0.40 kg/d, P < 0.01). At 7 d after birth, G1 calves had higher levels of haptoglobin (0.62 vs. 0.43 g/L, P < 0.02), ceruloplasmin (2.39 vs. 1.87 μmol/L, P < 0.01), and reactive oxygen metabolites (14.8 vs. 12.9 mg H_2O_2/100 mL, P < 0.03). These differences indicate that G1 calves (intake of IgG < 100 g with first meal) experienced important inflammatory events after birth, which increased the oxidative stress, impaired liver function, and strongly reduced the ADG. Therefore, to avoid these problems it is insufficient to only check first colostrum quality before feeding. Besides providing a better measure of the IgG intake, an evaluation of targeted blood parameters within 1 wk of life could give more detailed information on calf health and welfare.

Key Words: calves, colostrum, immunoglobulin, inflammation, metabolic profile

1147 Effect of the timing of addition of *trans*-10, *cis*-12 conjuaged linoleic acid and L-carnitine during culture on development and cryotolerance of bovine embryos produced in vitro.

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The objective was to determine whether embryo development and survival following cryopreservation were affected by the timing of addition of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) and L-carnitine during culture. Bovine embryos were produced in vitro using abattoir-derived cumulus-oocyte complexes. After fertilization, presumptive zygotes (n = 2804) were cultured at 38.5°C in a humidified atmosphere of 5% O_2, 5% CO_2, and 90% N_2 in synthetic oviductal fluid-bovine embryos 1 (SOF-BE1). Presumptive zygotes were randomly assigned to the following treatment groups: vehicle for the entire culture period, 100 μM CLA for the first 88 h, last 72 h, or the entire culture period and 0.75 mM L-carnitine for the first 88 h, last 72 h, or the entire culture period. At 88 h post-insemination, embryos were removed from culture, washed in HEPES-Tyrode's albumin lactate pyruvate, placed into their respective culture treatments and cultured for an additional 72 h. The proportion of oocytes that cleaved was assessed at 88 h (Day 4) after insemination and the proportion of oocytes that developed to the blastocyst and advanced blastocyst (expanded, hatching, and hatched) stages was determined on Day 7. Blastocysts and expanded blastocysts (n = 537) were harvested at Day 7 and subjected to controlled-rate freezing following equilibration in 1.5 M ethylene glycol. After thawing, embryos were cultured for 72 h in SOF-BE1 supplemented with 10% (v/v) fetal bovine serum and 50 mM dithiothreitol at 38.5°C in a humidified atmosphere of 5% O_2, 5% CO_2 and 90% N_2. Post-thaw re-expansion and hatching rates were determined at 24, 48, and 72 h. The experiment was replicated 14 times and data were analyzed by analysis of variance. There was no effect of treatment with CLA or L-carnitine on embryo development or post-thaw survival (Table 1.), regardless of whether treatment occurred during the first 88 h, the last 72 h or the entire culture period. While previous studies have reported beneficial effects of CLA and L-carnitine on embryo cryotolerance, the results of the present study indicate the effects of CLA and L-carnitine are likely not dependent on the timing of their addition during culture.

Key Words: conjugated linoleic acid, cryopreservation, L-carnitine,

1148 An insufficient supply of glucose substrates causes reduced lactose synthesis in lactating dairy cows fed cereal straws instead of alfalfa hay. B. Wang[*1], F. Zhao[1,2], B. X. Zhang[1], and J. X. Liu[1], *[1]Institute of Dairy Science, Zhejiang University, Hangzhou, China, [2]University of Vermont, Burlington.*

The objective of the present study was to investigate the nutrient supply for lactose synthesis in the mammary gland (MG) of lactating cows fed different sources of forage. Thirty Holstein cows were randomly assigned into three groups and fed 3 diets contained 30% corn stover (CS), 30% rice straw (RS), or 23% alfalfa hay plus 7% Chinese wild rye hay (AH) as forage sources, respectively, with identical total concentration and corn silage for 14 wk. Milk lactose, rumen VFA, blood glucose and hormones (n = 10), and mRNA abundance of genes related to glucose metabolism in liver and MG (n = 6) were analyzed in these cows. The data were analyzed using PROC MIXED program of SAS with a randomized complete block design with repeated measures. The percentage of milk lactose was always lower in the RS-fed cows than the cows fed AH or CS during the last 12 wk of feeding trial ($P < 0.01$). The ruminal propionate concentrations were also reduced in the RS group compared to the AH group ($P = 0.03$). The ratio of plasma insulin to glucagon in the mammary vein was greater in the AH group than in the CS or RS group ($P = 0.04$). The abundance of the pyruvate carboxylase mRNA in the liver was reduced in the RS group compared to the AH or CS groups ($P = 0.04$), whereas the mRNA abundance of mitochondrial phosphoenolpyruvate carboxykinase, insulin like growth factor-1 receptor, and phosphofructokinase-liver, -muscle, and -platelet in the liver was reduced in the RS group compared to the AH group ($P < 0.05$). The mammary glucose uptake was greater in the AH-fed cows than in the CS- or RS-fed cows ($P = 0.02$). The mRNA abundance of the glucose transporters in the MG was similar between the 3 treatments. The mRNA abundance of α-lactalbumin in the MG of the cows fed RS tended to increase compared to that of the cows fed AH or CS. The milk potassium concentration was increased in the cows fed RS compared to those fed AH or CS ($P < 0.01$). In summary, the insufficient ruminal propionate levels in the cows fed RS were associated with decreased gluconeogenesis in the liver, resulting in the shortage of the arterial glucose supply for mammary uptake and reduced lactose synthesis.

Key Words: cereal straw diets, glucose substrates, lactose synthesis

1149 Expression of genes involved in the initial steps of steroidogenesis in adipose tissue depots of dairy cows during the dry period and early lactation. A. Alizadeh[1,2,3], H. Sadri[1], J. Rehage[4], S. Dänicke[5], and H. Sauerwein[*1], *[1]Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Bonn, Germany, [2]Department of Animal Science, Saveh Branch, Islamic Azad University, Saveh, Iran, [3]Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran, [4]University for Veterinary Medicine, Foundation, Hannover, Germany, [5]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Braunschweig, Germany.*

In view of the significant changes in body fat content related to parturition and lactation in dairy cows, together with the role of adipose tissue (AT) not only as a store of steroid hormones but also as a potential site of steroidogenesis, mobilization of body reserves may also alter steroid hormone secretion and eventually reproduction. With this background, our research objectives were (1) to assess the expression of two rate-limiting factors of steroidogenesis, i.e., steroidogenic acute regulatory protein (StAR) and Cytochrome P450scc (CYP11A1) in bovine AT, (2) to characterize the time course of their mRNA abundance during late pregnancy and early lactation, and (3) to compare this time course in a subcutaneous versus a visceral fat depot. StAR triggers cholesterol delivery to the inner mitochondrial membrane (IMM) where CYP11A1 then initiates steroidogenesis by converting cholesterol to pregnenolone, the precursor of all other endogenous steroids. Biopsies were collected from 20 Holstein cows from the subcutaneous (sc) and the retroperitoneal fat depot (rp) on d −42 and d 1, 21, and 100 relative to calving. The mRNA abundance of StAR and CYP11A1 was assessed in the tissue samples by qPCR and normalized using the 4 most stable reference genes. Data were analyzed using the MIXED procedure of SAS. In scAT, the StAR mRNA abundance was lowest on d −42, increased on d 1 ($P < 0.05$) until d 21 to remain at this level at d 100 (~3-fold greater than on d −42; $P < 0.001$). Expression of StAR mRNA in rpAT increased with time of lactation, but differences between sampling days were limited to d −42 versus d 100 (3.3-fold increase; $P = 0.01$). Expression of CYP11A1 mRNA was not detectable in both AT with the protocol used herein (suitability of the protocol was confirmed with ovary as positive control). The increased expression of StAR mRNA abundance after calving in rpAT and scAT indicate an increased capacity for cholesterol uptake to the IMM. Given the apparently absent expression of CYP11A1, de novo synthesis of steroid hormones in bovine AT seems implausible and AT steroid metabolism likely depends on the uptake of preformed steroid precursors. Cholesterol reaching the IMM may thus rather be metabolized

to oxysterols which were suggested to have regulatory functions in adipocytes.

Key Words: adipose tissue, dairy cow, StAR

1150 Effects of a dietary supplementation of rumen-protected B vitamins on reproduction of dairy cows by measuring nutrigenomic parameters.

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It has been known that supplementary rumen-protected or injected B vitamins can improve dairy cow milk production and reproductive performance. Recently, it has been reported that B vitamins injections were having an impact on granulosa cells of the ovarian follicle when looking at gene expression profiling. Therefore, the aim of the present study was to assess whether rumen-protected B vitamins given as a dietary supplement can have an impact on gene expression in granulosa cells. The experimental design included 30 cows divided in three groups; 1) control, without any B vitamin supplementation, 2) injection, weekly intramuscular injections of 320 mg of folic acid and 10 mg of vitamin B12 starting from −21 to 60 d post calving, and 3) dietary supplementation of rumen-protected B vitamins as 50 g/cow/d of Transition VB™ (Jefo) from −21 to −1 calving, 100 g/cow/d of Transition VB™ from 1 to 21 d post-calving, 3 g/cow/d of Lactation VB™(Jefo) from calving to 60 d post calving. The follicles size was measured by laparoscopic transvaginal ultrasound from 40 d until OPU at 54 d post-calving. The cows were synchronized with two injections of PGF2α 12 d apart with first injection at 40 d. Granulosa cells were collected by OPU 53 h post second injection on the dominant follicle larger than 12 mm in diameter. Hybridization was done in dye swipe on EmbryoGENE microarray slides using three trios of animals. Differently expressed genes were analyzed through Ingenuity Pathway Analysis software. Selected genes were further assessed by RT-qPCR based on their functional significances. Based on estradiol and progesterone levels, the pattern of gene expression is supporting precocious granulosa cell differentiation toward an earlier response to LH (up-regulation of RGS2, NR3C1, OLR1, since significantly different ($p < 0.05$) from the control in both microarray analysis and RT-qPCR validation; downregulation of LHCGR, HSD3B1 and FST, since significantly different ($p < 0.05$) from the control) which may be the result of an increase in LH secretion. While comparing gene expression to superovulation conditions improving oocyte developmental competence, we observed genes commonly expressed with dietary supplementation of protected B vitamins (RGS2 and INHBA). The microarray data of granulosa cells from the dominant follicle are supporting the hypothesis that dietary supplementation of rumen-protected B vitamins is affecting granulosa cells differentiation toward an earlier LH response associated with genes expressed in conditions where oocyte developmental competence is improved.

Key Words: microarray, nutrigenomic, protected B vitamins, reproduction

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1151 Impact of dietary protein levels during late pregnancy on the number of binuclear cells in sheep.

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Angiogenic and vasoactive factors have been localized to binuclear cells (BNCs) located in the placenta of several species including sheep. During late gestation in the ewe, a low protein diet increased maternal blood pressure and uterine blood flow compared with a high dietary protein level. The objective of this study is to determine the effect of varying protein levels during late pregnancy in ewes on the number of BNCs. We hypothesized that low dietary protein during late gestation would increase the number of BNCs leading to the reported increase in uterine blood flow in ewes. At Day 100 of pregnancy, 18 ewes were randomly divided into three groups (6 each) and provided one of three diets containing different metabolizable protein (MP) levels: low protein level (L; 60% MP), control protein level (C; 100% MP), and high protein level (H; 140% MP). At Day 130 ± 1 of gestation dams were humanly euthanized and placentomes were removed for histology analysis. Histology sections were stained with biotinylated Dolichos biflurus (DBA) lectin, Texas red-avidin, and fluorescein (Fluorescien labeled Griffonia simplifolica lectin). There was no significant effect ($P = 0.90$) of maternal protein level on BNC number (180.0, 166.1, and 165.2 ± 28.41 for L, C, and H, respectively). Furthermore, there was no significant effect of maternal protein level on BNC size, proportion of the placentome occupied by cotyledon, nor number of BNCs per cotyledonary area. While we reject our hypothesis that BNC numbers are increased in protein deficient pregnant ewes, we will continue to evaluate if the ovine BNCs produce angiogenic or vasoactive factors that may influence placental function.

Key Words: binuclear cell, placentome, sheep

1152 Effect of serum concentration of β-carotene at AI on productive and reproductive parameters in lactating Holstein cows. A. M. L. Madureira*[1], T. Guzella Guida[1], R. L. A. Cerri[2], and J. L. M. Vasconcelos[1], [1]*Sao Paulo State University, Botucatu, Brazil,* [2]*Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada.*

The objective of this study was to determine the effect of β-carotene concentration in serum at the moment of artificial insemination (AI) on Holstein cows. A total of 497 lactating dairy cows were enrolled. All animals were assigned to a timed AI protocol (CIDR+ estradiol benzoate+GnRH-7d-PG-F-2d-CIDR-out+PGF+ECP-2d-timed AI). Blood samples and body condition score were collected at the moment of AI. The serum B-carotene was quantified in a single step denaturation and extraction into a solvent, followed by measurement using a portable spectrophotometer (iCheck; BioAnalyt, GmbH, Teltow, Germany). Milk production and herd health records were collected for the entire experimental period, and pregnancy diagnosis performed by ultrasound 31 d post-AI. Data were analyzed using the MIXED and GLIMMIX procedures of SAS. Animals with BCS $\leq$ 2.75 had lower ($P < 0.01$) concentration of B-Carotene compared with cows with BCS $\geq$ 3.0 (3.82 ± 0.09 μg/ml and 4.16 ± 0.06μg/ml, respectively). Multiparous cows had greater concentration of B-Carotene compared with primiparous ($P < 0.01$). The concentration of B-carotene at TAI was greater in cows with at least one disease episode between parturition and timed AI compared with healthy animals (3.95 ± 0.12 μg/ml vs. 5.12 ± 0.46 μg/ml). There was no correlation between milk production and concentration of B-carotene ($r < 0.01$), but a quadratic correlation between pregnancy per AI and concentration of B-carotene ($P = 0.03$) was found. When serum B-carotene was categorized as low (< 3.0 μg/ml), intermediate ($\geq$ 3.0– < 6.0 μg/ml) and high $\geq$ 6.0μg/ml, cows with intermediate concentrations were more fertile than the other two categories (29.0%; 38.9% and 22.6%, respectively; $P = 0.05$). In conclusion, the concentration of β-carotene was affected by BCS, parity, incidence of diseases. Animals with intermediate concentrations in serum had greater pregnancy per AI, suggesting a possible use as a marker for fertility in lactating dairy cows.

Key Words: β-carotene, fertility, pregnancy per AI

1153 Propionic acid decreased hepatic acetyl CoA content compared with glycerol within the timeframe of meals when infused abomasally. L. B. Gualdron-Duarte* and M. S. Allen, *Michigan State University, East Lansing.*

We previously reported that propionic acid (P) reduced dry matter (DMI) and metabolizable energy intake compared with glycerol (G) when administered as isoenergetic infusions to the abomasum of cows in the postpartum (PP) period. Our objective in this experiment was to evaluate short-term effects of P compared with G on hepatic acetyl CoA (AcCoA) content for PP cows. We hypothesized that P compared with G will decrease the AcCoA content in the liver within the time frame of a meal. Six ruminal cannulated cows in the PP period (15.2 ± 7.7 d PP) were used in a crossover design experiment to evaluate the effects of G and P pulse dosed to the abomasum on hepatic AcCoA content and plasma concentrations of metabolites and hormones. Cows were randomly allocated to treatment sequence (G-P and P-G) and each block was completed in 3 d with 2 collection days and a rest day between them. Two moles of P or G (2 mol/500mL, $\geq$ 99.5%) were dosed within 1 min to the abomasum 1 h before feeding. Liver tissue was biopsied and blood was collected immediately before dosing and at 30 and 60 min after dosing. Treatments interacted with time to affect AcCoA content ($P < 0.01$). At 30 min after dosing, P decreased AcCoA content by 34% while G increased AcCoA content by 32%, resulting in differences in AcCoA for P compared to G at 30 min (18.0 vs. 36.9 nM/g, $P < 0.0001$), which persisted at 60 min after dosing (21.9 vs. 32.8 nM/g, $P < 0.01$). Plasma BHBA concentration decreased and glucose concentration increased over time for both treatments ($P < 0.001$). While plasma NEFA concentration tended ($P = 0.059$) to be lower for P compared with G at 30 min, it was numerically higher by 60 min when hepatic AcCoA content was still lower. Therefore, the reduction of hepatic AcCoA content by P compared with G was likely because of oxidation in the tricarboxylic acid cycle. This is consistent with these treatment effects on DMI according to the Hepatic Oxidation Theory.

Key Words: acetyl CoA, hepatic oxidation, postpartum

1154 Feed restriction-induced negative energy balance alters the fatty acid profiles of adipose tissue and milk fat of dairy cows. S. E. Schmidt*, K. M. Thelen, C. L. Preseault, G. A. Contreras, and A. L. Lock, *Michigan State University, East Lansing.*

Negative energy balance (NEB) during early lactation results in extensive adipose tissue lipolysis in dairy cows. However, it is not clear if specific adipose depots or fatty acids (FA) are preferentially mobilized. Our objective was to characterize the FA profile of adipose depots and milk fat following feed restriction-induced NEB. Twelve multiparous late lactation (> 200 DIM) Holstein cows, in two experimental blocks, were subjected to treatments consisting of ad libitum feed intake (ADLIB; $n = 6$) or feed restriction (RESTR; $n = 6$) resulting in an energy balance of −13.3 ± 0.5 Mcal/d over 4 d. Milk samples were analyzed for FA composition and collected on d 4. Following the treatment period, all cows were slaughtered and tissue samples were collected from 6 adipose depots: omental, subcutaneous flank, tailhead, perirenal, inguinal, and sternal. Statistical analysis of adipose and milk FA composition was

performed using linear mixed models. RESTR increased the C14 desaturase index (*cis*-9 C14:1/(*cis*-9 C14:1 + C14:0)) of the sternal and tailhead depots ($P < 0.05$) and the C16 desaturase index (*cis*-9 C16:1/(*cis*-9 C16:1 + C16:0)) of the tailhead depot ($P < 0.01$). RESTR decreased C18:0 content of the tailhead depot (8.7 vs. 12.8 g/100 g FA; $P = 0.01$). Across all depots, RESTR increased the *cis*-9 C14:1 content of adipose tissue ($P = 0.04$). RESTR decreased daily yield of de novo-synthesized FA in milk ($P = 0.02$) but the yields of 16-carbon and preformed FA were not affected by treatment ($P \geq 0.20$). Compared to ADLIB, RESTR increased the C18 desaturase index (*cis*-9 C18:1/(*cis*-9 C18:1 + C18:0)) (0.73 vs. 0.66) and the C16 desaturase index (0.08 vs. 0.05) of milk fat ($P < 0.01$). RESTR increased the daily yields of *cis*-9 C16:1 and *cis*-9 C18:1 in milk fat compared to ADLIB (both $P < 0.05$), while the yields of C16:0 and C18:0 were not affected by treatment ($P \geq 0.24$). RESTR increased total monounsaturated FA yield in milk ($P = 0.03$), but treatment did not alter total saturated FA or polyunsaturated FA yields ($P \geq 0.11$). Alterations in adipose FA composition, suggesting mobilization of saturated FA, occurred only in the subcutaneous adipose depots, and most dramatically in the tailhead. However, increased desaturase activity in the mammary gland most likely prevented a subsequent increase in saturated FA yield of milk.

Key Words: adipose, energy balance, lipolysis

1155 Body condition score and body condition score change: Associations with fertility phenotypes in lactating dairy cows.

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The objective was to examine the associations between BCS and BCS change and utero-ovarian status in first and second parity dairy cows (n = 910; 22 commercial pasture-based dairy herds). Each cow was examined at wk 3 ± 1 and 7 ± 1 postpartum. Transrectal ultrasound exams were conducted to determine presence/absence of corpora lutea (CL). BCS was measured using a 1 to 5 scale, with increments of 0.25. Vaginal discharge score (VDS) of the contents of the vagina was assessed using the Metricheck device. For VDS, an objective scoring scheme on a 1 to 5 scale with 1 point increments was utilized (1 = no infection; 2 = mild infection; > 2 = severe infection). Binary data were analyzed using the GLIMMIX procedure of SAS. Dependent variables were CL and VDS at 3 and 7 wk postpartum. Independent variables were BCS and BCS change (loss, constant, or gain between 3 and 7 wk postpartum), with farm, parity, and DIM on the day of the exam included as adjustment variables. For CL, VDS score was included as an adjustment variable, whereas for VDS, CL was included as an adjustment variable. Fixed effects were tested and retained where $P \leq 0.1$. For variables with a binary distribution (CL) model-adjusted LSMEAN values are presented, whereas, for variables with a multinomial distribution (VDS), unadjusted raw mean values are presented. The proportion of cows that had a CL at wk 3 was 0.45 and 0.58 when cows had BCS $\leq$ 2.75 and $\geq$ 3.00, respectively ($P = 0.002$), and the corresponding figures at wk 7 were 0.74 and 0.89, respectively ($P < 0.0001$). There was no association between BCS change and CL at wk 3 ($P = 0.7$) or wk 7 ($P = 0.9$). At wk 3, the proportion of cows that were classified as having no infection, mild infection, or severe infection was 0.15, 0.23, and 0.62 for cows with BCS $\leq$ 2.75 and 0.15, 0.32, and 0.53 for cows with BCS $\geq$ 3.00, respectively ($P = 0.01$). At 6–8 wk, the proportion of cows that were classified as having no infection, mild infection, or severe infection was 0.46, 0.32, and 0.23 for cows with BCS $\leq$ 2.75 and 0.52, 0.30, and 0.18 for cows with BCS $\geq$ 3.00, respectively ($P = 0.05$). The findings highlight the importance of BCS during early lactation for restoration of cyclicity and uterine health status in pasture-based systems.

Key Words: BCS, utero-ovarian status

1156 Effects of Omnigen-AF supplementation on body temperature, milk production, and somatic cell count in lactating dairy cows.

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The objective this study was to evaluate the effects of Omnigen-AF (OMN; Phibro Animal Health, Teaneck, NJ) supplementation on body temperature, and production variables of lactating dairy cows. Thirty-two Holstein × Gir cows (100 ± 10 d in milk) were assigned to either control or OMN treated groups, balanced for previous milk production, parity, and body condition score. Dietary treatments were individually fed to cows once daily after the morning milking, at a rate of 56 g/day of kaolin (rumen-inert substance) or OMN added to 200 g of ground corn used as carrier for 56 d, and separated from the TMR diet that was fed ad libitum and formulated to meet or exceed cow nutritional requirements. Temperature and humidity loggers were used to record environmental data and temperature-humidity index. To assess body temperature, each animal was inserted with a thermometer coupled to an intravaginal device from d 15 to 28, and d 43 to 56 of the experiment. Thermometers were set to measure vaginal temperature every 10 min, and values were averaged hourly. Supplementation with OMN increased ($P < 0.01$) milk yield (21.3 vs. 20.1 kg/d for OMN and control, respectively; SEM = 0.13) and DMI (17.6 vs. 16.6 kg/d for OMN and control, respectively; SEM = 0.19), and decreased ($P = 0.06$) milk somatic

cells count (5.30 vs. 5.48 $\log_{10}$ cells/mL for OMN and control, respectively; SEM = 0.05). Treatment × time interactions were not significant ($P \geq 0.15$) for the production variables analyzed herein. Furthermore, when environmental temperature-humidity index was > 68, cows supplemented with OMN remained less ($P < 0.01$) time with body temperature above 39.1°C (38 vs. 45% for OMN and control, respectively; SEM = 0.05%). In conclusion, supplementing OMN to lactating dairy cows increased milk yield and DMI, decreased milk somatic cells count, and reduced incidence of rectal temperature above 39.1°C under heat stress conditions.

Key Words: dairy cows, dietary supplementation, heat stress

1157 The effects of stage of gestation and maternal nutrient status on binucleate cell numbers in the beef cow.

A. M. Peterson*[1], A. Reyaz[1], S. T. Dorsam[1], L. E. Camacho[2], K. C. Swanson[1], A. Grazul-Bilska[1], and K. A. Vonnahme[1], *[1]North Dakota State University, Fargo, [2]University of Arizona, Tucson.*

Our laboratory has demonstrated that maternal nutrition in beef cows can impact uterine blood flow and vascular function of excised placental arteries. However, there is little evidence to suggest that maternal nutrition is impacting capillary number. There is evidence in other species that multi-nucleated cells (i.e., binucleate cells; BNC) in the placenta may produce vasoactive factors. The objective was to test the hypothesis that maternal nutrient restriction followed by early realimentation would increase BNC numbers in the bovine placentome. On d 30 of pregnancy, multiparous, non-lactating cows (620.5 ± 11.3 kg) were assigned to 1 of 2 dietary treatments: control (C;100% NRC; $n = 18$) and restricted (R; 60% NRC; $n = 30$). On d 85, cows were slaughtered (C, $n = 6$; R, $n = 6$), remained on control (CC; $n = 12$) and restricted (RR; $n = 12$), or were realimented to control (RC; $n = 11$). On d 140, cows were slaughtered (CC, $n = 6$; RR, $n = 6$; RC, $n = 5$), remained on control (CCC, $n = 6$; RCC, $n = 5$), or were realimented to control (RRC, $n = 6$). On d 254, all remaining cows were slaughtered. Placentomes were collected and fixed in formalin. Tissue sections were stained using biotinylated lectin Dolichos Biflorus Agglutinin (DBA), Texas red avidin, and DAPI mounting media to visualize BNC. Using image analysis, BNC number, BNC size, and percentage BNC area per tissue area were determined. While there was no effect of diet ($P > 0.11$) on any measurement, there was a main effect of day ($P < 0.01$) where BNC numbers decreased as gestation advanced (572.5, 504.1, 489.1 ± 31.35 for d 85, 140, and 254, respectively). In addition, the BNC size decreased ($P < 0.01$) from d 85 to 140 of gestation, then increased until d 254 (63.1, 51.8, 65.2 ± 3.0 mm^2, respectively). Similarly, the percent BNC area per tissue area decreased ($P < 0.01$) from d 85 to 140 and then increased by d 254 (3.76, 2.78, 3.39 ± 0.20%, respectively). Thus, stage of pregnancy but not diet affected selected BNC measurements indicating a specific role of BNC as pregnancy progresses. Additional studies are currently underway to investigate expression of vasoactive factors (e.g., endothelial nitric oxide) by the bovine BNC, and if maternal diet can alter the expression of those factors.

Key Words: binucleate cell, cow, placentome

1158 Effects of post-AI supplementation with Ca salts of soybean oil on ovarian and pregnancy development in *Bos indicus* beef cows.

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The objective of this experiment was to compare corpus luteum (CL) and pregnancy development in beef cows supplemented or not with Ca salts of soybean oil for 21 d (CSSO) beginning after timed-AI. One hundred lactating multiparous Nelore (*Bos indicus*) cows (BW = 430 ± 5 kg, BCS = 2.87 ± 0.02; age = 8.5 ± 0.2 yr; days post-partum = 152 ± 1 d) were inseminated on d 0 of the experiment, and divided into 20 groups of 5 cows/group. Groups were randomly assigned to receive (as-fed basis) 100 g of protein-mineral mix + 100 g of ground corn per cow/d, in addition to: 1) 100 g/cow daily of CSSO ($n = 10$), or 2) 100 g/cow daily of kaolin (CON; rumen-inert indigestible substance; $n = 10$). Groups were maintained in 4 *Panicum maximum* pastures (5 groups from the same treatment within each pasture) with ad libitum access to forage. However, groups were segregated daily and offered treatments individually during the experimental period (d 0 to 21). Blood samples were collected and transrectal ultrasonography was performed to verify ovulation and corpus luteum (CL) volume immediately before AI (d 0), on d 7, and 15. Immediately after ultrasonography on d 15, 60 cows (30 cows/treatment, 3 cows/group) diagnosed without the presence of a CL on d 0, but with a CL greater than 0.38 cm^3 in volume on d 7 and 15, were assigned to embryo collection via uterine flushing with PBS. On d 30, final pregnancy status was determined via transrectal ultrasonography. No treatment differences ($P = 0.68$) were detected on dominant follicle diameter on d 0. However, mean CL volume on d 7 and 15 was greater ($P = 0.04$) for CSSO vs. CON cows (3.2 vs. 2.7 cm^3, respectively; SEM = 0.2). No treatment differences were detected ($P \geq 0.85$) for the proportion of cows that had an embryo on d 15 (40.0 vs. 39.2% for CSSO and CON cows, respectively; SEM = 8.9) or diagnosed as pregnant on d 30. However, embryos

collected from CSSO cows were longer ($P = 0.04$) compared with embryos collected from CSSO cohorts (2.57 vs. 1.15 cm, respectively; SEM = 0.59). In summary, supplementing beef cows with 100 g of CSSO beginning after AI increased CL and embryo development by d 15 of gestation.

Key Words: beef cows, Ca salts of soybean oil, embryo, ovary, pregnancy

PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: PRE- AND POST-NATAL IMPACTS ON OFFSPRING PERFORMANCE

1159 Consequences of early nutritional insults on fetal hepatic glucose metabolism and insulin action. S. R. Wesolowski*, *University of Colorado School of Medicine, Aurora.*

Pregnancies complicated by placental insufficiency or reduced maternal nutrient supply produce fetuses with intrauterine growth restriction (IUGR). These in utero insults expose the fetus to a reduced supply of glucose, amino acids, and, in some cases, oxygen. The fetus adapts to these reductions in nutrient supply by reducing insulin secretion, increasing counter-regulatory hormone levels, and developing coordinated tissue-specific adaptations in glucose metabolism. Data from our fetal sheep model of IUGR demonstrate an early activation of hepatic glucose production and increased hepatic gluconeogenic gene expression (*PCK1*, *G6PC*) that are sustained during a hyperinsulinemic-euglycemic clamp, thus demonstrating the development of hepatic insulin resistance. This is liver specific insulin resistance because the IUGR fetus has a robust increase in non-hepatic insulin-stimulated glucose utilization in peripheral tissues. While this early activation of glucose production in utero may be an important adaptive response to produce glucose for other glucose-consuming fetal tissues, uncontrolled and dysregulated hepatic glucose production has adverse consequences postnatally and is a major component to diabetes in humans. The early mechanisms driving dysregulated hepatic glucose production and insulin resistance in the fetal liver are not fully understood. We have found that the AKT protein is robustly phosphorylated in the IUGR liver in response to insulin, yet downstream FOXO1 phosphorylation and nuclear localization is increased. We also find that despite decreased nutrient supply, stress signals like AMPK are not increased in the IUGR fetal liver. In addition to increased glucose production, our recent data also demonstrate decreased mitochondrial oxidation in the IUGR fetal liver and a compensatory increase in hepatic glycolysis, intrahepatic lactate production and utilization, and altered substrate preference for reduced hepatic oxidative metabolism. This combination of metabolic adaptations by the fetal liver may be necessary to activate and sustain GPR. However, decreased hepatic mitochondrial function that persists postnatally may underlie the development of hepatic steatosis in offspring who were IUGR. Overall, understanding the endocrine and molecular pathways responsible for these early metabolic adaptations in the fetus, will allow for development of targeted strategies to improve liver function in the fetus and improve postnatal growth and performance and decrease risk for diabetes and metabolic disease later in life.

Key Words: fetus, metabolic adaptations, nutrition

1160 Alterations in uteroplacental hemodynamics during melatonin supplementation in sheep and cattle. C. O. Lemley*[1] and K. A. Vonnahme[2], [1]*Mississippi State University, Mississippi State,* [2]*North Dakota State University, Fargo.*

Compromised placental function can result in fetal growth restriction which is associated with greater risk of neonatal morbidity and mortality. Large increases in transplacental nutrient and waste exchange, which support the exponential increase in fetal growth during the last half of gestation, are dependent primarily on the rapid growth and vascularization of the uteroplacenta. We are examining maternal nutritional plane along with therapeutic supplements, such as dietary melatonin, which impact placental vascularization, blood flow, and fetal development. Using a mid- to late-gestation ovine model of intrauterine growth restriction ($n = 31$), we examined uteroplacental blood flow and fetal growth during supplementation with 5 mg of dietary melatonin per day. Maternal nutrient restriction decreased uterine artery blood flow, while melatonin supplementation increased umbilical artery blood flow compared to non-supplemented controls. Although melatonin treatment did not rescue fetal weight in nutrient restricted ewes; we did observe disproportionate fetal size and fetal organ development. Moreover, fetal uptake of branched-chain amino acids was partially rescued by dietary melatonin supplementation. Elevated fetal concentrations of melatonin may result in altered blood flow distribution during important time points of development. These specific melatonin responses on umbilical artery hemodynamics and fetal development may be partially mediated through vascular melatonin receptors. Recently, we examined the effects of supplementing Holstein heifers ($n = 20$) with 20 mg of dietary melatonin per day during the last third of gestation. Uterine artery blood flow was increased by 25% and total serum antioxidant capacity was increased by 43% in melatonin supplemented heifers versus non-supplemented controls. In addition, peripheral concentrations of progesterone were decreased in melatonin supplemented heifers vs. non-supplemented controls. Using an in vitro model, melatonin treatment increased the activity of cytochrome P450 2C, a progesterone inactivating enzyme, which was blocked by treatment with the melatonin receptor antagonist, luzindole. Elucidating the consequences of specific therapeutic supplements on the continual plasticity of placental function will allow us to determine the proper timing