PHYSIOLOGY AND ENDOCRINOLOGY: PREGNANCY, PLACENTATION, AND REPRODUCTIVE HEALTH IN RUMINANTS

0486 Bioinformatics analysis of mammary gland and liver transcriptome in response to an intramammary *Escherichia coli* lipopolysaccharide challenge in early-lactation dairy cattle.

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During mastitis, pathogens are detected by the receptors on the epithelial cells of the mammary tissue and an acute phase response activates the immune system to eliminate the pathogens. The liver is a central organ during inflammation and synthesizes the necessary components for immediate defense at the site of tissue damage. The objective of this study was to determine gene expression patterns in mammary and liver tissue in response to an intramammary *E. coli* lipopolysaccharide (LPS) challenge in early lactating dairy cattle. Fourteen Holstein cows were used. At ~7 d in milk, 7 cows served as controls (CTR) and 7 cows (LPS) received an intramammary *E. coli* LPS challenge (200 μg in sterile saline). For transcript profiling the mammary and liver tissue were sampled by biopsy 2 h after the challenge. A bovine oligonucleotide (70-mers) microarray with > 13,000 annotated sequences was used for profiling. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and a threshold of 1.5-fold change and a *P* value of 0.05 were considered to define differentially expressed genes (DEG). Bioinformatics analyses was conducted using the dynamic impact approach (DIA) and ingenuity pathway analysis (IPA; Ingenuity Systems, Inc.). In mammary tissue, a total of 189 DEG (20 downregulated, 169 upregulated) were observed in LPS vs. CTR cows. The most-impacted and activated KEGG pathways highlighted by the DIA analysis were NOD-like receptor signaling, RIG-I-like receptor signaling, apoptosis, cytosolic DNA-sensing, and chemokine signaling. The IPA analysis underscored the presence of 13 transcription regulators (2 downregulated, 11 upregulated) of which 4 upregulated (NF-kB, MYC, STAT3, and HIF1A) are key components of inflammatory response processes and are involved in cell apoptosis. In liver tissue, a total of 107 DEG (42 downregulated, 65 upregulated) were observed due to LPS. The DIA analysis highlighted the inhibition of Fatty acid elongation in mitochondria and activation of p53 signaling pathway. From IPA analysis, the most important upregulated transcription regulators (ZFP36, CEBPD, MYC, and CREM) are involved in the immune and inflammatory responses and are necessary to maintaining homeostasis of the organism during infection. Results suggest that within 2 h from intramammary LPS challenge the liver responds to stimuli and alters its transcriptome as a way to maintain homeostasis.

Key Words: system biology, mammary, liver, lipopolysaccharide

0487 The role of pH and progesterone on bovine uterine protein secretion in response to maternal recognition, interferon-tau.

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Low uterine pH, associated with high dietary protein and blood urea, reduces fertility in dairy cows. The objective of this in vitro study was to determine the direct effects of a low pH on bovine endometrial (BEND) cells expression of Mx1 and ISG-15 in response to embryonic maternal recognition, interferon-tau (IFNτ), in the presence or absence of progesterone (P_4). In 2 experiments, using BEND cells as a model, the effects of a low pH environment was examined to determine the production of 2 IFNτ stimulated proteins, ISG-15 and Mx1. Bovine endometrial cells were grown to 80% confluency and further incubated for additional 24 h in culture media containing no P_4 (0 M; Exp. 1) or P_4 (10^{-7} M, Exp. 2). To reduce the pH, in both experiments, cells (90% confluent) were treated with dimethyldioxirane (DMD), at final concentrations of 0, 10, 15, and 20 m*M* (pH of 7.35, 7.17, 6.9, and 6.76, respectively) and subsequently, challenged with 0 or 10,000 antiviral units of rIFNτ. Cells were incubated for an additional 24 h. Once harvested, BEND cells were lysed and supernatants were analyzed and quantified for Mx1 and ISG-15, using SDS-PAGE and Western Immunoblotting protocols. Based on optical density, at 0 mM DMD, regardless of P_4 treatment, IFNτ increased ($P < 0.01$) Mx1 and ISG-15 in both experiments. In Exp. 1 (P_4 free environment), there was effect of DMD ($P < 0.01$) and DMD by IFNτ interaction ($P < 0.01$) on both Mx1 and ISG-15. The 15 and 20 m*M* DMD reduced ($P < 0.01$) IFNτ- induced Mx1 expression, whereas only 20 m*M* DMD reduced ($P < 0.01$) ISG-15 expression in response to IFNτ. In Exp. 2, in presence of P_4, there was a significant effect of DMD and IFNτ by DMD interaction ($P < 0.01$) on Mx1 expression. However, there was no effect of DMD or DMD by IFNτ interaction on protein expression of ISG-15 ($P = 0.2$ and 0.4) in a P_4 environment. These results show that in absence of P_4 and low pH, IFNτ-stimulated proteins secretion are abrogated; however, P_4 overcame pH-induced decreased of ISG-15 but not Mx1. Progesterone may regulate the secretion of IFNτ- stimulated proteins in an acidic uterine environment.

Key Words: acidic pH, progesterone, interferon, bovine endometrial cells

0488 Hepatic steroid inactivating enzymes, hepatic portal blood flow, and corpus luteum blood perfusion in lactating dairy cattle. C. G. Hart*, B. E. Voelz, K. E. Brockus, and C. O. Lemley, *Mississippi State University, Mississippi State.*

In ruminants, a decrease in pregnancy rates may be due to decreased concentrations of progesterone (P4). It is important to note that both production from the corpus luteum and/or hepatic steroid inactivation impacts peripheral concentrations of P4. Cattle with an elevated dry matter intake have increased blood flow to the digestive tract and liver. This in turn leads to an increased delivery rate of steroids to the liver, and thus increased metabolism of these substrates. Excessive hepatic steroid inactivation contributes to decreased peripheral concentrations, which can alter reproductive performance. The objective of this study was to examine the activity of hepatic steroid inactivating enzymes in pregnant vs. nonpregnant lactating Holstein cows. Cows were synchronized using the Ovsynch plus CIDR (controlled internal drug release device) protocol and bred via artificial insemination on d 0. At d 10 post-AI, hepatic portal blood flow was measured via transabdominal Doppler ultrasonography. Images of corpus luteum blood perfusion were collected using the power flow program of the Doppler ultrasound. Blood perfusion was analyzed by examining pixel density using ImageJ software. Liver biopsies were collected and frozen for later determination of cytochrome P450 1A (CYP1A), 2C (CYP2C), 3A (CYP3A), and uridine diphosphate-glucuronosyltransferase (UGT) activities via luminogenic substrates. Aldo-keto reductase 1C (AKR1C) activity was measured using the specific substrate 1-acenaphthenol. Pregnancy was determined at d 33 of gestation and treatment groups were retrospectively assigned as pregnant or nonpregnant. Data were analyzed using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC). CYP1A, CYP2C, CYP3A, and AKR1C activity did not differ ($P > 0.10$) between pregnant and nonpregnant cows. Activity of UGT per kg of BW was increased ($P < 0.05$) in pregnant (60.1 ± 3.4 RLU·min^{-1}·kg^{-1}) vs. nonpregnant (50.6 ± 3.4 RLU·min^{-1}·kg^{-1}) cows. Blood perfusion of the corpus luteum did not differ ($P > 0.30$) between pregnant and nonpregnant cows. Absolute hepatic portal blood flow was increased ($P < 0.05$) in pregnant (997 ± 78 L/h) vs. nonpregnant cows (748 ± 78 L/h). Portal blood flow per kg of BW was increased ($P < 0.05$) in pregnant (1.65 ± 0.13 L/h/kg) vs. nonpregnant cows (1.27 ± 0.13 L/h/kg). The current study highlights the relevance of further investigation into steroid secretion and inactivation and their impact on the maintenance of pregnancy in dairy cattle.

Key Words: blood flow, cattle, steroid

0489 Effects of supplementing Holstein heifers with dietary melatonin during late gestation on growth and cardiovascular measurements of offspring. K. E. Brockus*, C. G. Hart, S. H. Ward, and C. O. Lemley, *Mississippi State University, Mississippi State.*

The objective was to examine the effects of supplementing dams with dietary melatonin during late gestation on offspring cardiovascular and growth measurements. On d 190 of gestation, heifers ($n = 20$) were blocked by BW and then randomly assigned to 1 of 2 dietary treatments: (1) 20 mg of dietary melatonin d^{-1} (MEL) or (2) no melatonin supplementation (CON). Dietary treatments were terminated on d 262 of gestation. At birth, calves were separated from their dams and given 3.8 L of colostrum. Calves were fed 5.7 L of whole milk daily and offered 0.9 kg/d of starter grain. Starter was increased by 0.9 kg/d when orts were 0 kg. Calf ($n = 18$) measurements of growth, blood pressure, and cortisol were collected on wk 0, 1, 2, 3, and 4 of age. Calf hepatic portal blood flow, determined by transabdominal Doppler ultrasonography, and concentrations of IGF-1 were determined on wk 0 and 4 of age. Dependent variables were analyzed using repeated-measures ANOVA of the mixed procedure of SAS (SAS Inst. Inc., Cary, NC) with the model statement containing treatment, age, and their respective interaction. Calf BW, abdominal girth, hip height, and wither height increased ($P < 0.05$) with age. An age by treatment interaction ($P < 0.01$) was observed for calf heart girth, which was decreased at wk 2 in calves from MEL treated heifers compared with calves from control treated heifers. Pulse pressure (41 ± 2 mm Hg), mean arterial pressure (90 ± 2 mm Hg), absolute hepatic portal blood flow (3137 ± 326 mL/min), and blood flow relative to body weight (74 ± 8 mL/[min × kg]) were not different ($P > 0.05$) between treatments. A main effect of calf age ($P < 0.05$) was observed for concentrations of IGF-1, which was decreased at wk 4 (9.0 ± 0.4 ng/mL) compared with wk 0 (10.8 ± 0.9 ng/mL). An age by treatment interaction ($P < 0.05$) was observed for concentrations of cortisol, which was decreased at wk 2 in calves from MEL treated dams (1.2 ± 0.8 ng/mL) compared with calves from CON treated dams (5.8 ± 0.8 ng/mL). Early postnatal growth and hepatic portal blood flow were not different in offspring born to dams supplemented with dietary melatonin. However, the difference in offspring concentrations of cortisol following maternal melatonin supplementation needs further investigation.

Key Words: cortisol, hepatic portal blood flow, melatonin

0490 Uterine blood flow, calf, and placental weights from beef cows supplemented during late gestation. V. C. Kennedy*, B. R. Mordhorst, M. L. Bauer, K. C. Swanson, and K. A. Vonnahme, *North Dakota State University, Fargo.*

Maternal nutrition impacts uterine blood flow (BF), thus offspring development. This study's objective was to investigate the effects of supplementing dried distiller's grains with solubles (DDGS) during late gestation on uterine BF, calf and placental weights. Multiparous beef cows were randomly divided into a control group (CON; $n = 15$) consuming a diet containing 90% corn stover and 10% corn silage (DM basis) ad libitum and a treatment group (TRT; $n = 12$) consuming the same diet and DDGS (0.3% BW). Corn silage inclusion was increased to 30% as gestation progressed. Intake was monitored and controlled via Insentec roughage feeders. Ipsilateral and contralateral uterine BF and cross-sectional area (CSA) at the bifurcation of each uterine artery was measured by Doppler ultrasonography on d 180, 216, and 246 of pregnancy. At parturition calves and placentas were weighed. Data analysis utilized the mixed procedure in SAS (SAS Inst. Inc., Cary, NC). Contralateral uterine artery BF and CSA increased ($P < 0.01$) as gestation advanced. For ipsilateral uterine artery BF and CSA, there was a treatment by day of gestation interaction ($P < 0.05$). The CSA was similar ($P = 0.30$) on d 181, but was greater ($P \leq 0.02$) in TRT vs. CON cows on d 216 and 246 (0.94 vs. 0.71 ± 0.07 cm^2 and 1.14 vs. 0.76 ± 0.07 cm^2, respectively). Ipsilateral BF tended to be greater ($P = 0.06$) on d 181 in TRT cows compared with CON (11.4 vs. 8.2 ± 1.1 L/min), and was greater on d 216 and 246 (21.1 vs. 15.4 ± 1.8 and 32.6 vs. 19.6 ± 2.7 L/min, respectively). There was no treatment by day interaction ($P = 0.17$) for total uterine BF, but there was a main effect of treatment ($P = 0.02$) and day ($P < 0.01$). DDGS cows had increased uterine BF compared with CON (25.5 vs. 19.1 ± 1.8 L/min). Total uterine BF increased ($P < 0.01$) as gestation advanced (12.2, 22.2, 32.7 ± 2.2 L/min for d 181, 216, and 246). While there was no effect of treatment on gestation length ($P = 0.43$) or placental weights ($P \geq 0.22$), there was a tendency ($P = 0.06$) for calves born to TRT cows to be heavier (43.3 vs. 40.5 ± 1.0 kg). Supplementation with DDGS increased uterine BF which contrasts with our previous study; protein and caloric intake differences between these studies is currently under investigation.

Key Words: beef cow, pregnancy, uterine blood flow

0491 Possible markers of uterine and metabolic health in transition dairy cows. G. Esposito*[1,2], A. Chapwanya[2], E. C. Webb[2,3], and P. C. Irons[1,2], *[1]Department of Production Animal Studies, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort, South Africa, [2]Institute of Food, Nutrition and Well-being University of Pretoria, Pretoria, South Africa, [3]Department of Animal and Wildlife Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa*.

In transition dairy cows negative energy balance (NEB) status commonly leads to perturbed fertility, reduced immune function and decreased milk yield. The objective of this study was to investigate the relationship between indicators of NEB, systemic inflammation postpartum, and genital diseases in transition dairy cows. Prepartum Holstein cows ($n = 10$), from 20 d before the predicted day of calving until 35 d in milk (DIM) were assigned to 2 treatments: NEB (80% net energy requirements) and control. Dry matter intake (DMI) was recorded daily and clinical evaluation was conducted once a week. From the day of calving, milk yield, somatic cells count (scc), lactose, fat, protein, and fat/protein ratio were recorded daily. From 7 till 35 DIM, weekly endometrial samples were collected for cytological evaluation, metagenomic characterization of the endometrial DNA and measurements of the expression of inflammatory genes. Ovarian activity was monitored every other day from 7 DIM. Blood samples were collected weekly and analyzed for NEFA, β-hydroxybutyrate (BHBA) and cholesterol. Correlationship between variables was evaluated using Spearman's rank correlation test. Moreover, a stepwise regression analyses was performed to explain variability of indicators of uterine and metabolic status. As expected, NEB cows showed higher DMI ($P < 0.1$), lower milk production ($P < 0.05$) and a higher fat:protein ratio ($P < 0.05$) until 35 DIM. In addition, NEFA and BHBA were higher in the NEB cows ($P < 0.05$), while cholesterol was lower ($P < 0.001$). Moreover, even if not strong, a negative correlation was observed between BHBA levels and increment in BCS (-0.37), and between NEFA and numbers of dominant follicles observed (-0.25). In addition, a negative correlation was observed between percentage of polymorphonuclear cells (PMS), from the endometrial samples, and serum cholesterol (-0.40) supporting our hypothesis that total cholesterol level could be one of the possible markers for uterine health evaluation. In addition, a positive correlation was observed between NEFA and PMS cells (-0.40). Furthermore, the stepwise regression analysis confirmed that serum BHBA and cholesterol levels were the ones that better explained the other variables (clinical evaluation, intake, reproduction). Data regarding the metagenomic characterization of the endometrial DNA and on the expression of inflammatory genes are still pending. The preliminary results confirmed the effects of NEB on biochemical parameters with potential as

predictors of metabolic and genital diseases. Further validation based on larger data sets is required.

Key Words: negative energy balance, transition cow, uterine involution

0492 Pregnancy-induced changes in metabolome and proteome in ovine uterine flushings. T. R. Hansen*, J. J. Romero, C. Broeckling, and J. E. Prenni, *Colorado State University, Fort Collins.*

The endometrium serves as a primitive placenta by secreting histotroph, which nourishes the developing conceptus (embryo proper and extraembryonic membranes). Modern mass spectroscopy (MS) approaches allow for global analysis of proteins and metabolites in bodily fluids. We hypothesized that global MS can identify metabolites and proteins that are induced by pregnancy in uterine flushings. To test this hypothesis, uteri were collected on d 12 of the estrous cycle (n = 5 ewes not exposed to ram) or d 12 (n = 4), 14 (n = 5), or 16 (n = 5) of pregnancy (confirmed by presence of conceptus) and flushed using physiological buffered saline. Pregnancy status and Day were main effects analyzed by GLM-SAS (SAS Inst. Inc., Cary, NC). Metabolites were extracted from uterine flushings using 80% methanol and profiled using UPLC-MS. The proteome was examined by digestion with trypsin, followed by analysis of peptides with LC-MS/MS. Metabolite profiling resulted in the detection of 8510 molecular features, of which 5 were confirmed to be upregulated (> threefold and $P < 0.05$) in response to pregnancy by d 14 to 16 and were not detected on d 12: acetylcarnitine, carnitine, ecdysteroids, N-acetyl dileucine, and valine. These metabolites function in fatty acid transport (carnitines), antiapoptotic mechanisms (ecdysteroids), and availability of nutrients (amino acids). Proteome analysis resulted in the detection of 783 proteins that were differentially regulated ($P < 0.05$) by d 14 to 16 of pregnancy, 7 of which are described herein: annexin A1, A2, and A5; calcium binding protein (S100A11); profilin 1, trophoblast kunitz domain protein 1 (TKDP), and interferon tau (IFNT). These proteins have unique functions in mediating endocytosis, exocytosis, calcium signaling, and inhibition of prostaglandins (annexins and associated S100A11); protecting against maternal proteases (TKDP); remodeling cytoskeleton (profilin 1); and altering uterine release of prostaglandin F2 α, as well as inducing interferon stimulated genes in the endometrium and the corpus luteum (IFNT). It is concluded that global MS approaches are poweful in delineating the metabolome and proteome in uterine flushings and identifying differential expression in response to the conceptus by d 14 to 16 of pregnancy. *USDA NIFA AFRI 2011–67015–20067.*

Key Words: pregnancy, uterus, conceptus, proteome, metabolome

0493 Syncytin expression in uterine endometrium and fetal membranes during early pregnancy in sheep. K. J. McLean*, L. P. Reynolds, A. Grazul-Bilska, J. Haring, and J. S. Caton, *North Dakota State University, Fargo.*

Endogenous retroviruses may be involved in formation of the placental interface between the endometrium and fetal membranes (FM). When this interface is not adequately formed, fetal loss or growth retardation may occur. Syncytin is an integrated retroviral envelope gene thought to be involved in cell-cell fusion and immunosuppression within the mammalian placenta; however, in sheep the exact function of syncytin is unknown. Ewes also possess ~20 copies of another endogenous retrovirus (enJSRV) which is closely related to the exogenous Jaagsiekte sheep retrovirus. Integration and expression of enJSRV strains, in the placenta, is breed specific. To examine syncytin and enJSRV expression at the maternal-fetal interface throughout early gestation, crossbred western whiteface (primarily Rambouillet, Targhee, and Columbia) ewes were naturally mated and gravid uteri were obtained on d 14, 16, 18, 20, 22, 24, 26, 28, and 30 (n = 6 to 8/d) after mating (day of mating = d 0). Nonpregnant, mid-luteal ewes (d 10; n = 8) were used as controls. Expression of syncytin and enJSRV-18 was determined with snap-frozen caruncular (maternal placental) tissue and FM (chorion on d16 and chorioallantois thereafter) using quantitative real-time RT-PCR. Statistical analyses used PROC GLM of SAS (SAS Inst. Inc., Cary, NC) with orthogonal contrasts. Fetal length increased threefold ($P < 0.001$) from d 20 (5.50 ± 0.97 mm) to d 30 (19.29 ± 0.52 mm) of gestation. Fetal membrane expression of syncytin decreased from d 16 thru d 20, was increased from d 22 until d 26, and decreased, again, to d 30 ($P = 0.002$). Both fetal growth patterns and FM expression of syncytin had significant linear ($P < 0.005$) and cubic ($P < 0.001$) orthogonal contrasts. Pregnant ewes had greater syncytin expression ($P = 0.002$) compared with nonpregnant ewes. Syncytin expression in caruncular tissues decreased from d 14 until d 20, then increased to d 24 and remained steady to d 30 of gestation ($P = 0.009$) resulting in quadratic ($P = 0.01$) and cubic ($P = 0.001$) orthogonal contrasts. Interestingly, neither FM nor caruncular tissues of western whiteface ewes expressed detectable levels of enJSRV-18. Therefore, syncytin but not enJSRV-18 is likely involved in the regulation of placental function and growth during pregnancy in western whiteface sheep. *Supported by USDA-NRI Grant 2007–01215 to LPR and ATGB.*

Key Words: early gestation, placenta, syncytin

0494 Effect of postpartum treatment with nonsteroidal anti-inflammatory drugs (NSAID) on reproductive performance and removal from the herd in dairy cattle through mid-lactation.

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Many studies have evaluated the use of postpartum NSAID treatment in dairy cattle, but few have monitored long-term responses to such treatments. To address this limitation in the literature, 153 multiparous dairy cattle were blocked by breed, dystocia, and twin births, and assigned to 1 of 3 treatments within 12 to 36 h after parturition. Treatments were 1 placebo bolus on the first day after parturition and 3 consecutive daily drenches of sodium salicylate (125 $g \cdot cow^{-1} \cdot d^{-1}$) beginning on the first day after parturition (SS); 1 bolus of the NSAID meloxicam (675 mg/cow) and 3 drenches of an equal volume of water (M); and 1 placebo bolus and 3 drenches of water (CON). As previously reported, milk production was increased in the first 9 wk of lactation by either NSAID treatment in cows experiencing dystocia, but in cows that calved normally, milk yield increased only after treatment with M. The objective of this analysis was to determine if NSAID treatment influenced reproductive performance and risk of removal from the herd in approximately the first half of lactation (up to 160 d in milk, DIM). Removal rate from the herd and time to pregnancy were evaluated by Cox regression proportional hazard analysis, and incidence of disease was tested by Fisher's exact test. Treatment, breed, dystocia, twin births, and their interactions did not ($P > 0.1$) affect time to pregnancy or first service pregnancy per AI (21.9%). A total of 33 cows left the herd during the period investigated, and M cows were ($P = 0.02$) removed from the herd at a slower rate than CON (AHR = 0.33, 95% CI = 0.13, 0.84), with no effect observed for SS ($P = 0.28$, AHR = 0.66, 95% CI = 0.30, 1.42). Treatment did not affect the risk of leaving the herd due to mastitis, low milk production, injury, lameness, or death (all $P > 0.1$). However, M tended to decrease the risk of culling due to other diseases—including respiratory disease, displaced abomasum, and suspected metabolic disorders—compared with CON ($n = 7$ CON vs. 1 M; $P = 0.06$). Furthermore, 4 of the CON cows in this category were removed from the herd before 19 DIM, whereas the M cow was removed at 149 DIM. These results indicate that in addition to elevated milk production, postpartum administration of M may have beneficial effects on dairy cow longevity.

Key Words: inflammation, NSAID, cull rate, reproduction

0495 Biology and molecular signatures of elongating preimplantation conceptuses in dairy cows.

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The objectives were to investigate changes in transcriptome of preimplantation conceptuses during the process of elongation and associated changes in the concentration of interferon-tau (IFN-τ) in utero. Lactating dairy cows ($n = 160$) had estrous cycles synchronized and were subjected to induced ovulation and timed artificial insemination (AI). The day of AI was considered study d 0. On d 15, uteri were flushed and IFN-τ concentration in fluid measured. Recovered conceptuses were classified based on morphology and length as ovoid (OV; 1 to 4 mm), tubular (TUB; 5 to 19 mm), and filamentous (FIL; 20 to 85 mm). A subsample of conceptuses from each group had mRNA extracted and subjected to transcriptome analysis using Affymetrix Gene Chip Bovine Array (8 OV, 17 TUB, and 17 FIL). The experimental design was considered a prospective cohort study with 3 independent groups. Continuous variables were analyzed by ANOVA using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) and fitting adequate data distribution. Microarray data were analyzed using Bioconductor software in R environment. Data were preprocessed using Gene Chip Robust Multi-Array function. Limma package was used to fit a linear model and adjust variances by empirical Bayes adjustment. Moderate *t* test was performed for all pairwise comparisons, and *P* values were adjusted for multiple testing using the Benjamini and Hochberg false discovery rate. Adjusted $P < 0.05$ and fold change > 1.5 characterized significant differences. Functional analyses were performed using Ingenuity Pathway Analysis. Concentration of IFN-τ in uterine flushing differed ($P < 0.05$) among all 3 groups and was lower for cows with OV conceptus, followed by those with TUB and then FIL conceptuses (13.7, 326.8, and 2544.7 ng/mL, respectively). Transcriptome analyses revealed the upregulation of 321 and downregulation of 345 transcripts in the transition from OV to TUB, and the upregulation of 249 and downregulation of 154 transcripts in the transition from TUB to FIL. A total of 1441 transcripts were differently expressed when OV and FIL conceptuses were compared. Differently expressed genes were associated significantly with cellular movement, cell-to-cell signaling and interaction, cellular assembly and organization, lipid metabolism, small molecule biochemistry, and molecular transport. In conclusion, differences in conceptus morphology and length were associated with distinct concentrations of IFN-τ in utero and remarkable changes in transcriptome of trophectoderm cells that elucidate important cellular events occurring during conceptus elongation.

Key Words: conceptus elongation, transcriptome, dairy cow

0496 Modulation of the immune system during postpartum uterine infection. C. G. Walker*[1], S. Meier[1], J. R. Roche[1], M. D. Mitchell[2], and C. Burke[1], [1]*DairyNZ, Auckland, New Zealand,* [2]*University of Queensland, Australia.*

Postpartum uterine infection is associated with lower fertility at both the time of infection and after the infection has resolved. The objective of this study was to characterize genome-wide DNA methylation and gene expression in the endometrium of dairy cows with subclinical endometritis. It was hypothesized that aberrant DNA methylation may be involved in the subfertility associated with postpartum uterine infection. Endometrial tissues were obtained at 29 d postpartum (n = 12) and Agilent 2-color microarrays were used to characterize transcription and DNA methylation profiles. Analyses revealed 1856 probes to be differentially expressed in animals with subclinical endometritis (SUI) compared with control cows ($P <$ 0.05, Storey Multiple testing correction). No significant associations among DNA methylation and gene expression were detected. Further analysis using GeneGo Metacore and Gene Set Enrichment Analysis identified several pathways and processes enriched in the comparison. Several pathways that are involved in the innate immune response were enriched in SUI cows. Consistent with activation of toll like receptors (TLR) by microorganisms present in the uterus, there was enrichment for the TLR signaling pathway including increased expression of the transcription factor NFKB1, the proinflammatory cytokines IL1A and IL1B, downstream chemokines, cytokines, and acute phase and antimicrobial proteins in the endometrium of SUI cows. Further, the chemokine signaling pathway was enriched in SUI cows, with increased expression of genes that attract cells of the innate immune system. Increased expression of IL-8 and CXCL6, chemotactic factors for recruitment of neutrophils along with the immune cell surface marker PTPRC in SUI cows is consistent with the greater number of polymorphonuclear cells present in the uterus of these cows. Several antimicrobial peptides (LAP, TAP, DEFB1, DEFB10, DEFB103B, DEFB7) and acute phase proteins including SAA3, LBP, and the complement gene CFB had greater expression in SUI cows. Gene expression profiles in cows with subclinical endometritis in this study indicate that the immune response is activated, potentially resulting in a local proinflammatory environment in the uterus. If this period of inflammation is prolonged it could result in tissue damage or failure to complete involution of the uterus that may create a suboptimal environment for future pregnancy.

Key Words: endometritis, gene expression

0497 Carryover effects of postpartum diseases on early conceptus development in dairy cows. E. S. Ribeiro*, L. F. Greco, G. C. Gomes, R. Cerri, W. W. Thatcher, and J. E. P. Santos, *Department of Animal Sciences, University of Florida, Gainesville.*

Postpartum diseases constitute one of the major problems affecting fertility in dairy cows. The objective was to investigate the carryover effects of postpartum clinical diseases on early embryo development. From calving to first artificial insemination (AI), prevalence of calving problems, metritis, mastitis, lameness, digestive, and respiratory problems were recorded for a group of 617 dairy cows. Cows had estrous cycles synchronized and were subjected to induced ovulation and timed AI. A total of 419 cows had their uterus flushed on d 6 after AI and the structures recovered were evaluated for fertilization and embryo quality. The remaining 198 cows had their uterus flushed on d 15 after AI and interferon-tau (IFN-τ) concentration in fluid was measured and the recovered conceptuses were evaluated for size and morphology. A subsample of conceptuses (n = 22) had mRNA extracted and subjected to transcriptome analysis using Affymetrix Gene Chip Bovine Array. The experimental design was considered a prospective cohort study with 2 independent groups (healthy vs. diseased). Continuous variables were analyzed by ANOVA and binary data by logistic regression using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) and fitting adequate data distribution. Microarray data were analyzed using Bioconductor software in R environment. Data were preprocessed using Gene Chip Robust Multi-Array function. Limma package was used to fit a linear model and adjust variances by empirical Bayes adjustment. Moderate t test was performed for all pairwise comparisons, and P values were adjusted for multiple testing using the Benjamini and Hochberg false discovery rate. Adjusted $P < 0.05$ and fold change > 1.5 characterized significant differences. Functional analyses were performed using Ingenuity Pathway Analysis. Cows that had at least 1 clinical disease from calving to first AI had reduced fertilization, smaller proportion of good quality embryos on d 6, smaller size of conceptus and smaller concentration of IFN-τ on d 15 compared with cows that did not have clinical diseases. Controlling for size of the conceptuses, transcriptome analysis resulted in 41 transcripts that were differently expressed. The gene with the greatest difference in expression was FAT/CD36, which is important for cell signaling during conceptus elongation. FAT/CD36 was downregulated in conceptus recovered from cows that had diseases compared with those recovered from cows that did not have disease. In conclusion, clinical diseases prior insemination were associated with reduced fertilization and compromised early embryo development.

Key Words: embryo, dairy cow, disease