675 Hormonal manipulation of progesterone before initiation of an Ovsynch protocol to increase ovulatory response to the first GnRH treatment in Holstein cows. P. D. Carvalho*, M. C. Wiltbank, and P. M. Fricke, *Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.*

Our objective was to evaluate the effect of decreasing progesterone (P4) before initiation of an Ovsynch protocol on ovulatory response to the first GnRH injection (G1) and pregnancies per AI (P/AI). Lactating Holstein cows (n = 800) were synchronized using a Double Ovsynch protocol [Pre-Ovsynch protocol (GnRH; 7 d, $PGF_{2\alpha}$; 3 d, GnRH) followed 7 d later by an Ovsynch-56 protocol (G1; 7 d $PGF_{2\alpha}$; 24 h, $PGF_{2\alpha}$; 32 h, GnRH)] to receive first timed artificial insemination (TAI; 80 ± 3 DIM) 16 h after the last GnRH treatment. Cows were randomly assigned to receive a half-dose of $PGF_{2\alpha}$ (12.5 mg dinoprost tromethamine) 2 d before G1 (TRT) or serve as untreated controls (CON). Data were analyzed by logistic regression using GLIMMIX and ANOVA with MIXED procedures of SAS. Overall, CON cows had greater ($P < 0.01$) P4 than TRT cows at G1 (4.2 vs. 2.1 ng/mL). Ovulatory response to G1 was greater ($P < 0.01$) for TRT vs. CON cows [81.9% (90/110) vs. 60.9% (70/115), respectively]. Luteal regression during the second Ovsynch protocol did not differ ($P = 0.33$) between treatments [15.2% (15/99) vs. 10.6% (11/104); TRT vs. CON]. At 32 d after TAI, P/AI did not differ ($P = 0.34$) between treatments [56.2% (223/397) vs. 52.8% (209/396); TRT vs. CON]. At 67 d after AI, P/AI also did not differ ($P = 0.56$) between treatments [50.8% (190/374) vs. 48.6% (179/368); TRT vs. CON]. Pregnancy loss from 32 to 67 d after TAI did not differ [10.0% (21/211) vs. 9.6% (19/198); TRT vs. CON; $P = 0.90$]. Overall, cows that ovulated to G1 had greater ($P = 0.02$) P/AI compared with cows that did not ovulate [58.2% (89/153) vs. 41.5% (27/65), respectively]. The increase in P/AI in ovulating cows (16.7%) and observed increase in ovulation (21%; TRT – CON) produced an expected increase of 3.5% in P/AI in TRT vs. CON; similar to the observed 3.4% difference. Thus, administration of a half-dose of $PGF_{2\alpha}$ 2 d before G1 during a Double Ovsynch protocol decreased P4 at G1 and increased ovulatory response. Larger studies are needed to determine if this modified protocol increases P/AI. Supported by USDA Hatch project 231440.

Key Words: fertility, timed AI, ovulation

676 Progesterone concentration at initiation of Ovsynch and a second prostaglandin $F_{2\alpha}$ treatment affect luteal regression and fertility to timed AI in lactating Holstein cows. P. D. Carvalho*, M. J. Fuenzalida, V. G. Santos, A. Ricci, M. C. Wiltbank, and P. M. Fricke, *Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.*

Our objective was to compare luteal regression and pregnancies per AI (P/AI) after timed AI (TAI) for dairy cows receiving the first GnRH injection (G1) of an Ovsynch protocol in a low (<1.0 ng/mL) or a high (≥1.0 ng/mL) progesterone (P4) environment. Lactating Holstein cows (n = 851) at first or second and greater AI service were randomized to receive an Ovsynch protocol (G1; 7d, $PGF_{2\alpha}$; 56h, GnRH; 16h, TAI) with $1PGF_{2\alpha}$ (PGF) treatment (1PG; n = 440) or a modified Ovsynch protocol (G1; 7 d, $PGF_{2\alpha}$; 24 h, $PGF_{2\alpha}$; 32 h, GnRH; 16 h, TAI) with 2PGF treatments (2PG; n = 411). Blood was collected at G1, the first PGF treatment, and the last GnRH treatment (G2) of the Ovsynch protocol for analysis of P4. To eliminate cows undergoing early luteal regression, cows (n = 153) with P4 < 1.0 ng/mL at the first PGF treatment were excluded from the analysis. Cows with P4 < 0.4 ng/mL at G2 were defined as undergoing complete luteal regression after PGF, whereas cows with P4 ≥ 0.4 ng/mL at G2 were defined as failing to undergo complete luteal regression after PGF. Data were analyzed by logistic regression using PROC GLIMMIX of SAS. Overall, more ($P < 0.01$) cows with high P4 at G1 underwent luteal regression after PGF compared with cows with low P4 at G1 (92.2% vs. 82.8%, respectively). As expected, luteal regression after PGF was greater ($P < 0.01$) for 2PG (96.3%) compared with 1PG (83.8%) cows. For cows with low P4 at G1, more ($P < 0.01$) 2PG cows underwent luteal regression after PGF (69.6% vs. 96.2%, 1PG vs. 2PG). As a result, 2PG cows with low P4 at G1 had more ($P < 0.01$) P/AI compared with 1PG cows with low P4 at G1 (35.3% vs. 57.3% for 1PG vs. 2PG cows). By contrast, for cows with high P4 at G1, although luteal regression was greater for 2 PG (91% vs. 97%; 1PG vs. 2PG; $P = 0.05$), P/AI did not differ (35.9% vs. 39.7%; 1PG vs. 2PG; $P = 0.39$). We conclude that a second PGF injection 24 h after the first during an Ovsynch protocol increased luteal regression for cows with low or high P4 at G1 and increased P/AI for cows with low P4 at G1. Supported by USDA NIFA Hatch project 231440.

Key Words: Ovsynch, progesterone, fertility

677 Effect of high or low P4 during ovulatory follicle development on fertility of dairy cows. Joao Paulo N. Martins*[1], Dongliang Wang[2], Nanheng Mu[2], Guilherme F. Rossi[3], Vinicius R. Martins[1], Ana Paula Martini[4], Gilson A. Pessoa[4], and J. Richard Pursley[1], *[1]Department of Animal Science, Michigan State University, East Lansing, MI, [2]Shuozhou Vocational and Technical College, Shuozhou City, Shanxi, China, [3]Department of Preventive Veterinary Medicine and Animal Reproduction, FCAV-UNESP, Jaboticabal, SP, Brazil, [4]Department of Large Animal Clinical Science, Universidad Federal de Santa Maria, Santa Maria, RS, Brazil.*

The objective was to determine the effect of high vs. low progesterone (P4) during early and late stages of the ovulatory follicle development in lactating dairy cows. Cows that ovulated a d 7 1st wave dominant follicle were assigned to treatments. Ovaries were manipulated to induce high (H) or low (L) circulating concentrations of P4 during 0 to 4 d (early stage) and/or 5 to 7 d (late stage) of the wave forming 4 treatments: H/H, L/L, L/H and H/L. Luteolysis was induced with PG on d 7 of the treatment period. Ovulation of the dominant follicle was induced with GnRH 56 h following PG. All cows received AI 16 h later (n = 559). Pregnancy was determined 35 and 56 d after AI by ultrasonography and 117 d (±3) and 194 d (±3) by detection of pregnancy-specific protein B (PSPB) in milk. Data were analyzed with chi-squared analysis.Pregnancy/AI 35 d post AI was 43.2, 51.1, 53.6, and 60.4 for HH, HL, LH, and LL, respectively, and was greater for LL than HH cows. However, there was no difference on P/AI 56 d (HH = 42.4%, HL = 46%, LH = 51%, and LL = 51.1%), 117 d (HH = 38.3%, HL = 45.6%, LH = 48.3%, and LL = 48.9%), and 194 d post AI (HH = 36.8%, HL = 43.9%, LH = 44.4%, and LL = 46.3%). Pregnancy losses between 35 and 56 d post AI was 1.8, 10, 4.9 and 14.5% for HH, HL, LH, and LL, respectively, and was greater for LL than HH. There was no difference in pregnancy losses between 56 and 117 d post AI (HH = 5.8%, HL = 0%, LH = 2.8%, and LL = 2.9%) and between 117 and 194 d post AI (HH = 0%, HL = 0%, LH = 5.9%, and LL = 3.1%). Percentage of cows with double ovulations to the last GnRH was greater in LL (49%) compared with HH, HL and LH (12, 33, and 34% respectively). Cows that had double ovulation after the last GnRH had greater P/AI 35 d (45 vs. 66%) and 56 d post AI (43

vs. 57%) compared with cows with single ovulation. Pregnant losses between 35 and 56 d after AI was greater in cows with double ovulation compared with cows with single ovulation (4 vs. 14%) and tended to be greater if the ovulations were on the same ovary. In summary, low P4 during development of the ovulatory follicle increased the percentage of cows with double ovulations and P/AI 35 d post AI, but decreased embryonic survival between 35 and 56 d post AI.

Key Words: follicle, fertility, progesterone

678 Effect of a second dose of prostaglandin F_{2a} during Double-Ovsynch on successful luteolysis and fertility. Giovanni M. Baez*[1], Rafael V. Barletta[1], Alessandro Ricci[1], Eduardo Trevisol[1], Jerry N. Guenther[1], Alvaro Garcia-Guerra[1], Beatriz O. Cardoso[1], Mateus Z. Toledo[1], João P. Ferreira[2], and Milo C. Wiltbank[1], *[1]University of Wisconsin-Madison, Madison, WI, [2]São Paulo State University, Botucatu, SP, Brazil.*

Lack of complete regression of the corpus luteum (CL) after prostaglandin F_{2a} (PGF) treatment may reduce fertility during timed AI (TAI) protocols. A total of 373 lactating Holstein cows (172 primiparous; 201 multiparous) were synchronized with Double-Ovsynch starting at 53 ± 3 DIM (GnRH-7d-PGF-3d-GnRH-7d-GnRH-7d-PGF-56h-GnRH-16h-TAI). At the final PGF, cows were randomized to 1 of 2 treatments: 1PGF = No additional PGF treatment; 2PGF = Second PGF treatment 24h after first PGF. Blood samples were collected at the final PGF and GnRH treatments (72 and 16 h before timed AI). Only cows with P4 above 2.0 ng/ml before PGF (n = 344) were further analyzed. Fisher's exact test and *t*-test were used to analyze categorical and continuous variables respectively, and logistic regression analysis was used to calculate probabilities of CL regression and P/AI. Primiparous cows had greater P4 than multiparous cows (7.7 ± 0.22 vs. 6.5 ± 0.18; $P = 0.0001$) before treatment. At 56 h after PGF, P4 was greater ($P = 0.005$) for 1PGF (0.4 ± 0.04) than 2PGF (0.2 ± 0.05) cows. The percentage of cows with complete CL regression (<0.5 ng/mL at 56 h after PGF) was increased by second PGF for primparous (81.2% vs. 97.5%; 1PGF vs. 2 PGF; $P = 0.001$) or multiparous (84.4% vs, 96.7%; $P = 0.006$) cows. Cows with lower P4 at time of PGF had a reduced probability of complete CL regression with 1PGF by logistic regression ($P = 0.02$) or by comparing ($P = 0.0016$) quartile 1 (lowest P4; 2.0 to 4.8 ng/mL; 66.7%; 28/42) to the other 3 quartiles (88.1%; 118/134) which did not differ. In contrast, 2PGF cows had elevated CL regression (>95%) regardless of P4 at time of PGF ($P = 0.60$). Nonetheless, 2PGF increased CL regression for cows in both quartile 1 (66.7% vs. 95.1%; $P = 0.0016$) or quartiles 2–4 (88.1% vs. 97.6%; $P = 0.0034$). Interestingly, there was increasing P/AI with increasing P4 before PGF treatment for 2PGF ($P = 0.02$), but not 1PGF cows ($P = 0.13$). Cows with lower P4 at the time of PGF (Quartile 1) had similar P/AI for 1PGF (31.0%; 13/42) or 2PGF (31.7%; 13/41). In contrast, for quartiles 2–4 there was a tendency for decreased P/AI ($P = 0.10$) in 1PGF (44.0%; 59/134) vs. 2PGF (52.8%; 67/127) cows. Thus, treatment with a second PGF during Double-Ovsynch reduced inadequate CL regression and tended to increase P/AI, particularly in cows with greater P4 at the time of PGF treatment.

Key Words: prostaglandin F_{2a}, fertility, luteolysis

679 Differentially expressed genes in endometrium and corpus luteum of Holstein cows selected for high and low fertility are enriched for sequence variants associated with fertility. Stephen G. Moore*[1,2], Jennie E. Pryce[3], Ben J. Hayes[3], Amanda J. Chamberlain[3], Kathryn E. Kemper[3], Donagh P. Berry[1], Matthew McCabe[4], Paul Cormican[4], Patrick Lonergan[2], Trudee Fair[2], and Stephen T. Butler[1], *[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland, [2]University College Dublin, School of Agriculture and Food Science, Dublin, Ireland, [3]Department of Economic Development, Jobs, Transport and Resources & Dairy Futures Cooperative Research Centre (CRC), Agribio, La Trobe University, Bundoora, Australia, [4]Teagasc, Animal and Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland.*

In this study, we combined RNA-sequence data, genome-wide association studies (GWAS) and imputed sequence data (Daetwyler et al., 2014; Nat. Genet. 46: 858–865) to identify variants associated with dairy cow fertility. We tested the hypothesis that genes differentially expressed in the endometrium and corpus luteum (CL) on d 13 of the estrous cycle between cows with either good (Fert+) or poor (Fert-) genetic merit for fertility, would identify quantitative trait loci (QTL) regions and sequence variants associated with fertility in cattle. After an adjustment for multiple testing (Benjamini and Hochberg, $P \leq 0.05$), 9 and 560 genes were differentially expressed in the endometrium and CL, respectively, between Fert+ and Fert– cows. These differentially expressed genes (DEG) identified 93 QTL regions ($P < 10^{-3}$) that were validated by fertility GWAS using high-density genotypes from independent dairy cattle populations in both Australia (16,794 bull and cow genotypes) and Ireland (2,660 bull genotypes), with 54% of the signals detected primarily on BTA18 (23%), 5 (9%), 7 (8%), 8 (8%) and 29 (6%). The QTL regions were primarily associated with genes involved in prostaglandin $F_{2\alpha}$ (synthesis, secretion and action) and immune-related processes in the endometrium and CL. In addition, genes involved in steroidogenesis and mRNA processing were also identified in the CL. Seventeen sequence variants significantly associated with fertility ($P \leq 10^{-5}$) in the Australian population were identified within 2 kb upstream and downstream of DEG involved in mRNA processing or the immune system. One missense variant (SIFT value = 0.01; i.e., deleterious to protein function) significantly associated with fertility was identified in *EIF4EBP3*, a gene involved in translation initiation. The results of this study enhance our understanding of (1) the contribution of the endometrium and CL transcriptome to phenotypic reproductive performance; and (2) the genomic architecture influencing a complex trait such as dairy cow reproductive performance.

Key Words: fertility, corpus luteum, endometrium

680 Liver metabolism in dairy cows during repeated short-term feed-restrictions and LPS induced systemic inflammation. Josef J. Gross*[1], Emmanouil Kalaitzakis[2], Olga Wellnitz[1], Heiner Bollwein[2], and Rupert M. Bruckmaier[1], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Clinic of Reproductive Medicine, Vetsuisse Faculty University of Zurich, Zurich, Switzerland.*

Body fat mobilization during a negative energy balance (NEB) requires metabolic adaptations on the part of the liver. We investigated if responses of hepatic mRNA abundancies of genes involved in metabolism and immune system to an energy deficiency differ between lactational stages. Holstein dairy cows (n = 14) (control (CON) and restricted (RES) group) were fed with grass and additional concentrate from wk 3 ap until wk 12 pp, except the RES group receiving only grass during 1-wk feed-restrictions in wk 2, 5, 8, and 11 pp. At the end of the first restriction period, LPS from *E. coli* was infused intravenously (0.5 µg/kg BW) to induce an inflammatory status. Energy balance (EB) was calculated on a weekly basis. Blood was obtained weekly and liver tissue was collected for biopsy before and after restriction periods and at 8h after the systemic LPS challenge. Blood samples were analyzed for

glucose, NEFA, and BHBA concentrations. Hepatic gene expression of 3-hydroxybutyrate dehydrogenase (BDH) 2, carnitine palmitoyltransferase (CPT) 1A and 2, mitochondrial glycerol-3-phosphate-acyltransferase (GPAM), 3-hydroxy-3-methylglutaryl-coenzyme A synthase (HMGCS) 2, cytosolic phosphoenolpyruvate carboxykinase (PEPCKc), growth hormone receptor (GHR) 1A, insulin-like growth factor (IGF) 1, Hp (haptoglobin), serum amyloid A (SAA), and tumor necrosis factor (TNF) α were measured. Data were analyzed using a mixed model including group and wk as fixed effects. During restriction periods, RES had a more distinct negative EB (wk 2: Δ17 MJ NEL/d; wk 5: Δ19 MJ; wk 8: 6 MJ; wk 11 Δ13 MJ) and in wk 5 higher NEFA (0.64 mmol/L) and BHBA (0.65 mmol/L) concentrations compared with CON (0.39 and 0.37 mmol/L, resp.; $P < 0.05$). Hepatic mRNA abundances showed expression patterns depending on the lactational stage. During feed-restriction periods, expression of IGF-1 was upregulated in RES compared with CON (based on ratios between RES and CON: +11.1 in wk 2, +14.7 in wk 5, +2.9 in wk 8, +7.3 in wk 11; $P < 0.05$). At 8h after the initiation of the systemic LPS challenge, SAA, TNFα and Hp were downregulated in RES and CON, while GPAM, HMGCS2, GHR1A, CPT1A and 2, PEPCKc, and BDH2 were upregulated ($P < 0.05$). In conclusion the experiment showed that hepatic metabolism was clearly affected by feed-restrictions and systemic LPS-challenge.

Key Words: feed restriction, metabolism, dairy cow

681 Prediction of portal and hepatic blood flow in cattle. Jennifer L. Ellis*[1,2], Christopher K. Reynolds[3], Les A. Crompton[3], Mark Hanigan[4], Andre Bannink[5], James France[2], and Jan Dijkstra[1], *[1]Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands, [2]Centre for Nutrition Modelling, Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada, [3]School of Agriculture, Policy and Development, University of Reading, Reading, UK, [4]College of Agriculture and Life Science, Virginia Tech University, Blacksberg, VA, [5]Animal Nutrition, Wageningen UR Livestock Research, Wageningen, the Netherlands.*

An integral part of linking a multi-organ post-absorptive model is the prediction of nutrient fluxes between organs via blood flow. This paper reports a multivariate meta-analysis approach to model portal vein blood flow (PORBF) and hepatic venous blood flow (HEPBF) simultaneously. The developmental database consisted of 296 measurements (pAH dilution) for growing and lactating cattle with 55 treatments from 17 studies, and a separate evaluation database with 31 treatment means from 8 studies. Both databases had information on feed intake, bodyweight and diet composition. Blood flows predicted with DMI or metabolizable energy intake (MEI) was tested with both linear and quadratic equations using the NLINMIX macro of SAS. Cow(study) and study were treated as random effects and blood flow location (PORBF or HEPBF) as a repeated effect. Equations based on DMI rather than MEI typically resulted in higher concordance correlation coefficient (CCC) values, indicating better predictions. Quadratic equations did not out-perform their linear counterparts (CCC analysis), and quadratic equation terms were frequently non-significant. The best predictive equations were: PORBF (L/d) = 4855(±1097) + 2007(±74.8) × DMI (kg/d) and HEPBF (L/d) = 4463(±1094) + 2492(±74.5) × DMI (kg/d), with CCC values of 0.887 and 0.922, respectively. The residuals (predicted – observed) for PORBF expressed as a fraction of HEPBF (PORBF/HEPBF), and hepatic arterial blood flow (ARTBF (L/d); = HEPBF – PORBF) were affected by the proportion of forage in the diet, and thus equations for PORBF and HEPBF based on forage and concentrate DMI were developed: PORBF (L/d) = 5043(±1186) + 1989(±148.8) × Forage (kg DM/d) + 1989(±141.4) × Concentrate (kg DM/d), and HEPBF (L/d) = 4416(±1177) + 2223(±145.5) × Forage (kg DM/d) + 2741(±137.5) × Concentrate (kg DM/d), where CCC values were 0.886 and 0.912, respectively. The CCC for ARTBF improved from 0.877 to 0.904 and PORBF/HEPBF from 0.115 to 0.447 with forage and concentrate DMI separation. Developed equations predicted blood flow well, and also suggest different sensitivity of PORBF and HEPBF to the downstream effects of DMI composition.

Key Words: blood flow, liver, meta-analysis

682 Effects of reducing dietary cation-anion difference level on plasma Ca concentration and VDR expression level in gastrointestinal tract of transition mice. Wen-xuan Wu*, Hai-liang Xin, Yi Yang, and Ruo-yu Liu, *College of Animal Science, Guizhou Province, Guiyang, Guizhou Province, China.*

The mechanism of reducing dietary cation-anion difference (DCAD; $Na^+K–Cl^–S$, mmol/kg DM) to prevent hypocalcemia is not completely known. Therefore, this study was conducted to clarify the mechanism why reducing DCAD was effective for increase in plasma Ca concentration and thus for hypocalcemia prevention by detecting plasma Ca concentration and vitamin D receptor (VDR) expression level in gastrointestinal tracts (GIT) of transition mice. One hundred twenty transition mice were randomly allocated to 3 blocks with each of 40 individuals and were fed 3 diets with varying DCAD level at +300 (treatment 1, HD), +150 (control, CON), and −150 (treatment 2, LD), respectively. Ten mice for each treatment were killed to collect blood sample for plasma Ca concentration analysis and harvest GIT tissues (stomach, duodenum, jejunum, ileum, cecum, and colon) samples for VDR expression level detection at 4 time points: day of 20 (−20) and 5 (−5) before kidding, day of kidding (0), and day of 3 after kidding (+3). Data on plasma Ca was analyzed using SAS 9.3 with Proc MIXED and VDR mRNA with Proc NPar1 Way. Plasma Ca concentration (mg/dL) in LD mice (6.37) was higher than that in HD (4.43) and CON (4.98) mice for the whole and individual blood sampling time ($P < 0.05$). On d −20 diet LD resulted in higher VDR mRNA expression level in jejunum, ileum, and colon over diet HD ($P < 0.05$). On d −5 mice fed LD diet had more VDR mRNA expression level in jejunum and colon relative to HD and CON ($P < 0.05$). On d 0 feeding of treatment 2 had the highest VDR mRNA expression level in duodenum and colon, which was statistically higher than HD and CON ($P < 0.05$). On d 3 duodenum, jejunum, and colon were observed to show increased VDR mRNA expression level for LD compared with HD and CON ($P < 0.05$). These results indicated that reducing the DCAD upregulates VDR mRNA expression in GIT of transition mice and increases plasma Ca concentration, which explains some of the benefits of low DCAD on prevention of hypocalcemia.

Key Words: dietary cation-anion difference, plasma Ca, gastrointestinal VDR expression