PHYSIOLOGY AND ENDOCRINOLOGY III

1429 (W189) Estimated energy balance of periparturient ewes grazing in rangelands. E. González-García[*1], D. Tagliatella[2], M. Jouven[3], and F. Bocquier[3], *[1]INRA UMR868 Systèmes d'Elevage Méditerranées et Tropicaux (SELMET), Montpellier, France, [2]Universidade Estadual de Londrina (UEL), Rodovia Celso Garcia Cid, Campus Universitário, Londrina, Brazil, [3]Montpellier Supagro, Sciences Animales, UMR868 Systèmes d'Elevage Méditerranées et Tropicaux (SELMET), Montpellier, France.*

In a previous work we demonstrated that efficiency in body reserves (BR) mobilization/accretion was affected by parity [multiparous (MULT) ewes being more flexible than primiparous], litter size and physiological stage (peaks of BR mobilization attained around lambing and 1 mo after mating). The objective of this study was to estimate the dynamic individual energy balance of periparturient MULT Romane grazing ewes, from 15 d before until 15 d after lambing. A group of MULT ewes (n = 20), allocated according to litter size (lambing and suckling singletons, SING-n = 10-, or twins, TWIN-n = 10) was used. Details on management and feeding were reported by González-García et al. (2014). At late pregnancy, ewes were in rotational grazing of native rangeland and supplemented with 0.7, 2.0 and 0.8 kg/d of hay (*Dactylus glomerata* and alfalfa), silage (*Lolium perenne* and alfalfa) and barley, respectively. After lambing, ewes were fed on fertilized paddocks without supplementation. Individual progression of BW, BCS, plasma NEFA as well as ADG of lambs was considered for energy balance interpretation. Some estimation is established based on NRC (2007) recommendations. Data were analyzed using the PROC MIXED of SAS (2007) with repeated measures. During the last 4 wk of gestation, one 50 kg ewe from this flock is estimated to display a daily consumption of around 1.6 kg of DM (3.2% BW) to support around 180 g of BW gain, requiring 3.4 mcal of ME. During the first 6–8 wk lactation, feed intake is affected by litter size (NRC, 2007; 2.1 or 2.4 kg of DM/d for ewes suckling SING or TWIN; 4.2 or 4.8% BW, respectively) with an increase in energy requirement of 4.9 or 5.6 mcal of ME for SING or TWIN, respectively. At late pregnancy, a positive energy balance of > 1.1 mcal/d was observed (4.7 mcal of ME vs. 3.6 of ME requirements) due to the advantageous supplementation regime established in the farm. Paradoxically, at this stage (late pregnancy) NEFA values showed a peak in BR mobilization. After lambing, ewes suckling SING and TWIN were both required to mobilize their BR to meet energy requirement despite the high quality of the fertilized paddocks and the BW increase. More precise and targeted studies are required to better address the combined anabolic and catabolic phases experimented under the conditions of this experiment in periparturient ewes. Reference: González-García E. et al. (2014). Domestic Animal Endocrinology 46:37–48.

Key Words: periparturient ewes, rangelands, energy balance, body reserves

1430 (W190) Effects of adsorbent on milk aflatoxin M1 and lactation performance of dairy cows exposed to long-term challenge of aflatoxin B1. J. L. Xiong[1], Y. M. Wang[*2], W. M. Huang[1], Y. Zhang[1], H. M. Guo[1], and J. X. Liu[3], *[1]Institute of Dairy Science, Zhejiang University, Hangzhou, China, [2]Novus International Trading Co., Ltd, Shanghai, China, [3]Zhejiang University, Hangzhou, China.*

The objective of the study was to evaluate the effects of adsorbent on milk aflatoxin (AF) M1 and lactation performance of dairy cows exposed to long-term challenge of AFB1. Forty dairy cows were blocked based on days in milk (33 ± 7; mean ± SD) and milk production (33.9 ± 3.1 kg; mean ± SD), and were randomly assigned to one of four treatments in a 2 × 2 factorial arrangements with AFB1 (0 or 20 μg/kgDM) and Solis Mos (Novus International Inc., 0 or 0.25% of DM). The experiment lasted 9 wk, with the first week for adaptation. Milk yield and milk composition were recorded weekly, and serum concentrations of biochemical and antioxidant variables were analyzed in the first and the last week of the experiment. Milk AFM1 was analyzed by HPLC-MS/MS. Variables of data were analyzed using the PROC MIXED of SAS. Dry matter intake, milk yield, contents of milk protein and milk fat, and linear somatic cell count averaged 23.9 kg/d, 35.5 kg/d, 2.9%, 3.6%, and 5.1, respectively and were not affected (P > 0.05) by either AFB1 or Solis Mos supplement. Addition of Solis Mos in AFB1-contaminated diet significantly reduced (P < 0.01) milk AFM1 concentration (0.19 vs. 0.13 μg/kg) and transfer rates (1.38 vs. 0.89%). Dairy cows fed AFB1-contaminated diet had lower level of superoxide dismutase activity, total antioxidant capacity, glutathione peroxidase, IgG and IgA (P < 0.05), and higher level of malondialdehyde in plasma (P < 0.05). Inclusion of Solis Mos into diets increased the plasma superoxide dismutase activity, total antioxidant capacity, and IgG, while deceased malondialdehyde (P < 0.05). Neither AFB1 nor Solis Mos affected (P > 0.05) the plasma levels of alanine transaminase, aspartate aminotransferase, and alkaline phosphatase and IgM. It is concluded that inclusion of Solis Mos did not affect lactation performance, but reduced milk AFM1 concentration and transfer rate, and increased antioxidant capacity and immunity in early-lactating dairy cows exposed to long-term challenge of AFB1.

Key Words: adsorbent, aflatoxin, transfer

1431 (W191) Effects of excessive energy intake and supplementation with chromium propionate on insulin resistance parameters in lactating dairy cows: II. Glucose tolerance tests and follicular flushing. T. Leiva[1], R. F. Cooke[2], F. N. Correa[1], A. C. Aboin[1], A. P. Brandao[1], H. F. Soares[1], M. B. Piccolo[1], and J. L. M. Vasconcelos[*1], *[1]UNESP-FMVZ, Botucatu, Brazil, [2]Oregon State University-EOARC Burns, Burns.*

The objective of this experiment was to compare insulin resistance parameters and reproductive outcomes in lactating dairy cows with adequate or excessive energy intake, as well as in lactating dairy cows with excessive energy intake receiving Cr-propionate supplementation. Seventeen primiparous and multiparous, lactating Holstein cows were ranked by parity, BW, and BCS, and assigned to 1 of 3 dietary treatments on d 0: 1) diet to meet their NE_l requirements without Cr supplementation (MAN; $n = 5$), 2) diet to exceed their NE_l requirements without Cr supplementation (HIGH; $n = 6$), and 3) HIGH with 2.5 g/d of Cr-propionate (HIGHCR; $n = 8$, with 10 mg of Cr/cow daily). Cows were maintained in a single group and offered corn silage for ad libitum consumption, but received a corn-based concentrate twice daily via individual self-locking head gates from d 0 to 210. Concentrate intake was formulated to provide 100% of daily NE_l requirements of MAN and 160% of daily NE_l requirements of HIGH and HIGHCR cows. Glucose tolerance tests (GTT) were performed on d 40, 82, 124, 166, and 208 by infusing cows with 0.5 g of glucose/kg of BW, whereas blood samples were collected at -15, 0, 10, 20, 30, 45, 60, 90, and 120 min relative to infusion for determination of serum insulin and glucose. Follicle aspiration for in vitro embryo production was performed 2 d after each GTT. No treatment effects were detected ($P = 0.53$) for serum glucose concentrations. Treatment x min interactions ($P < 0.01$) were detected for serum insulin and insulin:glucose ratio, given that these parameters were greater ($P \leq 0.05$) for HIGH compared with HIGHCR and MAN from 10 to 60 min relative to glucose infusion, but always similar between HIGHCR and MAN ($P \geq 0.25$). A treatment x parity interaction was detected for oocyte collection ($P = 0.05$). Within multiparous cows, HIGHCR had greater ($P \leq 0.03$) number of viable oocytes collected compared with HIGH and MAN, whereas the same outcome was not detected ($P \geq 0.36$) within primiparous cows. No treatment effects were detected ($P \geq 0.33$) for number of embryos produced, or oocyte collected:embryo produced ratio. In conclusion, Cr-propionate supplementation prevented the increase in insulin resistance caused by excessive energy intake in lactating dairy cows during a GTT, and increased the number of viable oocytes collected during follicle aspiration for in vitro embryo production.

Key Words: chromium, dairy cows, energy, insulin resistance

1432 (W192) Deuterium enrichment in plasma, rumen fluid and urine of growing sheep dosed with D_2O intravenously and intraruminally does not differ. C. C. Metges[*1], S. Görs[1], H. M. Hammon[1], U. Agarwal[2], and B. J. Bequette[2], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Dep. of Animal and Avian Sciences, University of Maryland, College Park.*

The D_2O method has been used in humans to measure fractional gluconeogenesis. The advantage of this method is that all contributions of gluconeogenic substrates are considered. In ruminants, we aimed to determine whether the route of D_2O administration affects equilibration of deuterium with protons from water in various body water pools. Four sheep (23.5 ± 1 kg BW), equipped with a rumen fistula and a jugular vein catheter, were fed a pelleted ration (35 g/kg BW and d; 9 MJ ME/d) at 2-h intervals. Water was offered ad lib. To label body water, sheep were given two boli of 7 g D_2O/kg BW (99.2 atom% D) at 800 and 1200 h either into the rumen (IR) or into the jugular vein (IV) in a balanced crossover design. Two weeks separated each site of administration. Plasma was sampled before and hourly for 11 h following the first bolus whereas rumen fluid and urine were collected before and at 3, 6, 9, and 11 h. Samples were diluted and D_2O enrichments were measured by isotope ratio mass spectrometry. Paired *t* test was used to evaluate route effect. D_2O enrichments did not differ with route of tracer administration. A quasi-plateau in D2O enrichment was reached 2 h after the first bolus (IR: 0.76; IV: 0.78 atom% excess (APE)) with a further increase to a second plateau 2 h after the second bolus (IR: 1.48; IV: 1.47 APE; $P > 0.1$). Urine D_2O enrichment 3 h after the initial IR dose tended ($P = 0.09$) to be lower than with the IV route (IR: 0.47; IV: 0.78 APE), however, both routes of dosing lead to a similar maximum enrichment 9 to 11 h after the initial bolus (IR: 1.51; IV: 1.52 APE; $P > 0.1$). Rumen fluid D_2O enrichment attained a plateau 6 h after the initial bolus (IR: 1.51; IV: 1.43 APE; $P > 0.1$). This study verified that for measurement of fractional gluconeogenesis using the D_2O method, the kinetics of D_2O labelling are similar with the IR and IV routes of administration, and that either approach can be employed in ruminants.

Key Words: gluconeogenesis, deuterium oxide, rumen

1433 (W193) Manipulated plasma insulin, glucose, and BHBA affect immune factors in somatic cells in milk with and without intramammary LPS challenge in dairy cows. M. Zarrin[*1,2,3], R. M. Bruckmaier[1], and O. Wellnitz[1], *[1]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland, [2]Dep. of Animal Science, Yasouj University, Iran, [3]Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland.*

Changes of plasma hormones and metabolites affect mammary immunity during onset of lactation. Somatic cells are important for the initial udder defense and they are additionally recruited into milk during the immune response. This study aimed to investigate effects of long term (56 h) elevated insulin at simultaneous hypoglycemia or euglycemia, and elevated β-hydroxybutyrate (BHBA) concentrations, and additional intramammary LPS challenge on mRNA abundance of immune factors in somatic cells in milk. Animals were subjected to four intravenous treatment groups: an insulin infusion (HypoG, $n = 5$) to decrease plasma glucose concentration to 2.5 ± 0.1 mmol/L, a hyperinsulinemic euglycemic clamp to maintain plasma glucose concentration at pre-infusion level (EuG, $n = 6$), a BHBA infusion (HyperB, $n = 5$), and a 0.9% NaCl infusion (Control, $n = 8$). Two udder quarters were challenged with 200 µg *E.coli* LPS at 48 h of infusions. Cells were extracted from milk of control and treated quarters obtained before, after 48 h, and at the end of infusion with a quarter milking device. The mRNA abundances of immune factors were measured by RT-qPCR. Changes of mRNA abundance between before and after 48 h of infusions, and before and after LPS challenge were evaluated by analysis of variance with treatments as fixed effect. In HypoG mRNA abundance of interleukin (IL)-1β and RANTES (regulated on activation, normal T cell expressed and secreted) decreased ($P < 0.05$) during 48 h. In HyperB the mRNA abundance of IL-1β, IL-8, and RANTES tended to increase ($P < 0.1$) during 48 h. Intramammary LPS challenge up-regulated mRNA abundance of IL-1b, IL-8, and nuclear factor kappa-light-chain-enhancer of activated B cells after 8 h in all treatment groups ($P < 0.05$), and tumor necrosis factor-α in HypoG ($P < 0.05$). The mRNA abundance of IL-1β and IL-8 was up-regulated in HypoG ($P < 0.05$), and IL-1β, IL-8, and RANTES was downregulated in control quarters of HyperB ($P < 0.05$) 8 h after LPS challenge. Results demonstrate that intravenous insulin infusion downregulates the expression of immune factors in somatic cells in milk during hypoglycemia, whereas induced hyperketonemia seems to up-regulate some of these factors during 48 h. It can be speculated that the downregulation of immune factors in HypoG is related to a lack of energy (glucose) for the immune system, while BHBA is an alternative energy source for the immune system during immune response. Up-regulation of immune factors after LPS challenge was predictable, whereas mechanisms of downregulation in control quarters after LPS challenge are unclear.

Key Words: immunity, metabolite, LPS

1434 (W194) Effects of road transportation on metabolic and immunological responses in dairy heifers. M. Baik[*1], H. J. Kang[1], I. K. Lee[1], M. Y. Piao[1], C. W. Kwak[1], M. J. Gu[1], C. H. Yun[1], H. J. Kim[1], G. H. Kim[2], S. K. Kim[2], and J. K. Ha[1], *[1]Dep. of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, South Korea, [2]Dep. of Animal Science and Technology, Konkuk University, Seoul, South Korea.*

This study was performed to determine the effects of road transportation on metabolic responses in dairy heifers. Twenty-two Holstein heifers (average 17.6 mo of age, 440 kg of average body weight) were divided into non-transported (NT; $n = 8$) and transported (T; $n = 14$) groups. Feed and water in the NT heifers were restricted the same amount as the T heifers. The heifers were acclimated in stanchion barn. All heifers were restrained with a halter in a stanchion, and blood was collected using vaccutainer by jugular venipuncture. The NT heifers In the T group, blood was collected before transportation (BT), after 100 km (T1) and 200 km transportation (T2), and at 24 h after transportation (AT). In the NT group, blood was collected at same time as the T group. The T heifers showed higher ($P < 0.001$) blood cortisol concentrations after T1 and T2 than the NT heifers. The T heifers showed higher ($P < 0.01$) serum non-esterified fatty acid (NEFA) concentrations after T1 and T2 than the NT heifers. In contrast, the T heifers showed lower serum triglyceride (TG) concentrations after T1 ($P = 0.01$) and T2 ($P < 0.001$) than the NT heifers. Serum concentrations of cortisol, NEFA, and TG at 24 h AT were returned ($P > 0.05$) to those of the BT in the T heifers. Other serum lipid concentrations, including phospholipid ($P = 0.02$), high density lipoprotein ($P = 0.03$), low density lipoprotein ($P = 0.01$), and cholesterol ($P = 0.04$) were lower in the T heifers after T2 than the NT heifers. Serum glucose concentrations were not changed by T1 and T2. The ratio of granulocytes to lymphocytes ($P < 0.001$) and the percentage of monocytes ($P < 0.05$) were shown higher after T2 in the T heifers when compared to those of the NT heifers, suggesting increased number of innate immune cells on transportation stress. In conclusion, short transportation increases cortisol secretion, which was coincident with induction of metabolic responses and up-regulation of peripheral innate immune cells in dairy heifers.

Key Words: transportation, stress, metabolic responses, dairy heifers

1435 (W195) Differences in mitochondrial DNA copy numbers in various subcutaneous and visceral fat depots of overconditioned cows. L. Laubenthal[*1], L. Locher[2], J. Winkler[3], U. Meyer[3], J. Rehage[2], S. Dänicke[3], H. Sauerwein[1] and S. Häussler[1], *[1]University of Bonn, Institute of Animal Science, Germany, [2]University for Veterinary Medicine Foundation, Hannover, Germany, [3]Institute of Animal Nutrition, Friedrich Loeffler Institute (FLI), Braunschweig, Germany.*

In dairy cows, adipose tissue (AT) is mobilized during early lactation to meet the increased energy demands through lactation. Overconditioned cows are more prone to metabolic disorders during this period than lean cows. Just like in other tissues, mitochondria are the main site of energy production within AT. Different energy demands may lead to changes of mitochondrial DNA (mtDNA), which reflects the abundance of mitochondria in a cell. Different bovine AT depots differentiated into visceral (vc) and subcutaneous (sc) regions, might present different mtDNA contents, due to diverse metabolic functions. Therefore, we aimed to compare the number of mtDNA copies per cell between various sc and vcAT depots from overconditioned cows. Eight non-lactating, non-pregnant German Holstein cows (age 4 to 6 yr) received diets with increasing proportions of concentrate feed during the first 6 wk of the trial until 60% were reached. This diet was maintained for 10 wk and cows had an average body weight (BW) gain of 243 ± 33.3 kg within this period. Animals were slaughtered at the end of the experiment and tissue samples from sc (sternum, withers and tailhead) and vc (mesenterial, omental and retroperitoneal) AT were collected and snap frozen for genomic DNA isolation. The number of mtDNA copies/cell was quantified by multiplex quantitative PCR using β-globin as reference gene. Data (mean ± SEM) were analyzed using Mann–Whitney-U-test and the Spearman (r) correlation coefficient (SPSS). The number of mtDNA copies/cell was 2.6-fold higher in all vc compared to all sc ($P < 0.001$) AT. Retroperitoneal AT exhibits greatest mtDNA copies/cell (3488 ± 190) compared to all other AT depots. The mtDNA copy number/cell in mesenterial and omental AT were 3058 ± 405 and 2921 ± 235, respectively. In tailhead and sternum AT mtDNA copy number/cell was three-fold and in withers AT two-fold lower compared to retroperitoneal. Different amounts of mtDNA copies/cell might reflect individual energy demands and metabolic functions in different sc and vcAT depots. In this study, mtDNA was isolated from whole AT including both adipocytes and the cells belonging to the stromal vascular cell fraction (SVF). Therefore, lower values of mtDNA copies in scAT might be due to an increased SVF, which contains significantly less mtDNA copy numbers. Higher amounts of mtDNA copies per cell in vcAT compared to scAT are in accordance to the higher metabolic activity of vcAT, particularly the retroperitoneal AT depot.

Key Words: adipose tissue, mtDNA copy number, cows

1436 (W196) In vitro insulin sensitivity of subcutaneous and omental adipocytes of precalving dairy cows across a range of BCS. J. De Koster[*1], L. Hulpio[1], V. Fievez[2], W. Van den Broeck[3], and G. Opsomer[1], *[1]Dep. of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium, [2]Dep. of Animal Production, Faculty of Bioscience Engineering, Ghent University, Belgium, [3]Dep. of Morphology, Faculty of Veterinary Medicine, Ghent University, Belgium.*

The adipose tissue of dairy cows plays an important role during the transition period. Recent research indicates a selective mobilization of adipose depots during negative energy balance. In the present study, we investigated the effect of the size of the adipocytes and type of adipose depot (omental versus subcutaneous) on insulin sensitivity of the lipolytic activity of adipocytes from precalving dairy cows. Ten pregnant dairy cows (BCS 2.75–5) were euthanized and samples of subcutaneous (at the tail base) and omental adipose tissue were transferred to sterile falcon tubes containing medium (Krebs ringer bicarbonate hepes + 3% BSA). Adipose tissue fragments were minced and approximately 100 mg adipose tissue explants were incubated in 3 mL medium at 38°C on a shaker. Five different culture conditions were tested in duplicate: lipolytic activity in all the plates was stimulated with 10^{-6} mol/l isoproterenol and five different insulin concentrations (0; 1; 10; 200; 1000 μU/ml) were added. After 3 h of incubation, media were sampled for glycerol analysis. Results are expressed as nmol glycerol release per 3 h per million adipocytes. The number and volume of adipocytes were determined as described by DiGirolamo et al. (1971). Insulin decreased glycerol release and insulin sensitivity was expressed as percentage decrease of the maximal glycerol release (10^{-6} mol/l isoproterenol; 0 μU/ml insulin). Dose response curves were created using PROC NLIN in SAS to determine maximal effect and insulin dose needed to elicit halfmaximal effect (logED50). Effects on both parameters were analyzed using PROC MIXED in SAS with cow as random factor, adipose depot and volume of adipocytes as fixed factors. One cow was excluded because insulin failed to inhibit lipolytic activity. The maximal effect and logED50 for the insulin sensitivity were 0.32 ± 0.1279% and -0.22 ± 0.415 μU/ml, respectively in subcutaneous adipocytes and 0.23 ± 0.148% and 0.15 ± 0.849 μU/ml in omental adipocytes (mean ± stdev), respectively. Statistical analysis revealed negative effects of volume of the adipocytes (β = -0.2569; $P < 0.05$) and adipose depot (β = -0.2145; $0.1 < P < 0.05$, subcutaneous depot as reference) on the maximal effect while no significant effects were found on the logED50. Insulin sensitivity of the lipolytic activity of adipocytes in precalving dairy cows is influenced by the size of the adipocytes and the adipose depot with a lower maxi-

mal effect in omental adipocytes and lower maximal effect in larger adipocytes. The dose to elicit halfmaximal effect is not influenced by adipocyte size or adipose depot.

Key Words: in vitro insulin sensitivity, adipose depot, dairy cow

1437 (W197) Dietary melatonin supplementation during late gestation alters concentrations of progesterone and milk yield in Holstein heifers. C. O. Lemley*, K. E. Brockus, C. G. Hart, and S. H. Ward*, Mississippi State University, Starkville.*

The objective was to examine the effects of supplementing dietary melatonin during late gestation Holstein heifers on maternal concentrations of progesterone and estradiol-17β as well as subsequent milk yield during the first 30 d of lactation. On d 190 of gestation, heifers (n = 20) were blocked by body weight and then randomly assigned to one of two dietary treatments: 1) 20 mg of dietary melatonin per day (MEL) or 2) no melatonin supplementation (CON). At 0800 h, MEL heifers received 0.7 kg of grain top dressed with 2 mL of 10 mg/mL melatonin in ethanol while CON heifers received 0.7 kg of grain top dressed with 2 mL of ethanol alone. A TMR was provided after grain consumption. Supplementation ceased on d 262 of gestation for both treatment groups. Blood samples were collected on d 180 (baseline), 210, 240, and 262 of gestation. Serum concentrations of progesterone and estradiol-17β were determined via radioimmunoassay. Milk yield was recorded for the first 30 d of lactation. Dependent variables were analyzed using repeated-measures ANOVA of the PROC MIXED of SAS with the model statement containing dietary treatment, day, and their respective interaction. Main effects of dietary treatment or day are discussed in the absence of significant ($P < 0.05$) treatment by day interactions. Serum progesterone was decreased ($P < 0.05$) by 12% in MEL vs. CON heifers. Moreover, concentrations of progesterone were decreased ($P < 0.0001$) on d 262 of gestation vs. d 180, 210, and 240. Serum estradiol-17β tended to be decreased ($P = 0.06$) by 19% in MEL vs. CON heifers. Concentrations of estradiol-17β increased ($P < 0.0001$) as gestation proceeded. Gestation length was not different ($P > 0.50$) between treatments and averaged 275 ± 2 d. Daily milk yield showed a treatment by day interaction ($P < 0.01$), whereby milk yield was increased by 41% and 33% on d 2 and 3 of lactation in MEL vs. CON heifers, respectively. Dietary melatonin supplementation during late pregnancy altered steroid synthesis and/or clearance. In addition, the decreased concentrations of steroids during late gestation in MEL heifers had no adverse effects on subsequent milk yield during early lactation.

Key Words: melatonin, pregnancy, progesterone

1438 (W198) Dry-matter intake level and its effects on follicle growth and circulating progesterone in Nellore (*Bos indicus*) and Holstein (*Bos taurus*) heifers. E. O. S. Batista*[1], R. V. Sala[1], M. D. D. V. Ortolan[1], E. F. Jesus[2], T. A. D. Vale[3], G. G. Macedo[1], F. P. Rennó[3], A. H. Souza[4], and P. S. Baruselli[5], *[1]USP, São Paulo, Brazil, [2]School of Agricultural and Veterinary Sciences of UNESP, Jaboticabal, Brazil, [3]USP, Pirassununga, Brazil, [4]University of California–Davis, Davis, [5]University of Sao Paulo-VRA, Brazil.*

The aim of this study was to evaluate circulating progesterone concentration (P4) and ovarian follicular dynamics in *Bos indicus* and *Bos taurus* heifers under high (HDM; weight gain of 900 g per d) and low (LDM; maintenance, NRC 2001) consumption of dry matter. Cycling Holstein (n = 16) and Nellore (n = 16) heifers were used in a 2 × 2 factorial arrangement (crossover). The experimental diet was given during 32 d (15 d before and 17 d during the hormonal protocol). The animals were pre-synchronized with two applications of cloprostenol (0.53mg, i.m. $PGF_{2\alpha}$, Sincrocio, Ourofino Agronegócio) 12 d apart and 18 and 12 h before device insertion. At onset of synchronization protocol (d 0), heifers received a new intravaginal P4 device (CIDR, Zoetis Brasil), 2 mg of estradiol benzoate i.m. (BE, Sincrodiol, Ourofino Agronegócio) and a dose of $PGF_{2\alpha}$. After 8 d, the P4 device was removed and 1 mg of BE was administered 24 h later. Ultrasonographic exams were performed every 24 h during P4 device treatment (d 0 to 8), and at every 12 h from P4 device removal to ovulation. Blood samples were collected daily from d 0 to 10. The results were analyzed using PROC MIXED of SAS 9.2 and presented as mean ± standard error. An interaction (genetic group*d, $P < 0.0001$; diet*d, $P = 0.03$) was observed between genetic group, diet and d of the hormonal protocol on the diameter of the dominant follicle (DDF). The effect of diet on the DDF during hormonal protocol was observed as soon as two d after follicular wave emergence (d 5 to 10 of the hormonal protocol, $P < 0.05$). However, the effect of genetic group was observed only towards the end of the protocol (d 8 to 10, $P < 0.05$). Regardless of the diet, the DDF on d 8 ($P = 0.04$) and d 10 ($P = 0.01$) of the hormonal protocol were larger in Holstein (11.6 ± 0.4 and 14.4 ± 0.4) than Nellore (10.3 ± 0.4 and 12.2 ± 0.4) heifers. Independently from genetic group, the DDF on d 8 ($P < 0.0001$) and d 10 ($P = 0.01$) of the hormonal protocol was larger in heifers receiving to HDM (12.2 ± 0.4 and 14.3 ± 0.4) than LDM (9.6 ± 0.4 and 12.2 ± 0.4). Curiously, lowering DM intake caused a greater increase in circulating P4 in Nellore than in Holsteins (genetic group*diet*d, $P = 0.05$). In conclusion, heifer breed had a market effect in hormonal profile and follicle growth during synchronization programs, but increasing DM intake greatly influenced ovarian dynamics and circulating P4. *Acknowledgements: FAPESP, CNPq.*

Key Words: *Bos indicus, Bos taurus*, progesterone

1439 (W199) Association between insulin signaling and oxidative stress in serum and subcutaneous adipose tissue of overconditioned cows. S. Häussler[*1], L. Locher[2], L. Laubenthal[1], S. P. Singh[1], U. Meyer[3], J. Rehage[2], S. Dänicke[3], and H. Sauerwein[1], *[1]University of Bonn, Institute of Animal Science, Germany, [2]University for Veterinary Medicine Foundation, Hannover, Germany, [3]Institute of Animal Nutrition, Friedrich Loeffler Institute, Braunschweig, Germany.*

Cows with higher BCS or greater BCS loss in early lactation have more problems to adapt to the needs of lactation and are more sensitive to oxidative stress. Mitochondrial (mt)DNA copy numbers, reflecting the abundance of mitochondria in a cell, can increase to compensate mtDNA damage. Moreover, reactive oxygen species produced through lipid-induced mitochondrial dysfunction impair insulin signaling. We hypothesized that decreasing insulin sensitivity in overconditioned cows will be associated with oxidative stress concomitant with increased numbers of mitochondria. Therefore, we aimed to investigate the association between oxidative stress (assessed by quantifying derivates of reactive oxygen species (dROM)) and mtDNA copies/cell in subcutaneous (sc) adipose tissue (AT) with variables describing insulin sensitivity (IS) in overconditioned cows independently from homeorhetic processes. Non-pregnant, non-lactating German Holstein cows (n = 8) were gradually adapted to a high-energy ration (corn-grass-silage with increasing portion of corn-silage and increasing concentrate feed from 0% up to 60% of total dry matter intake). Over a period of 15 wk, the average weight gain of the animals was 243 ± 33.3 kg. Blood samples were collected once per month and were analyzed for insulin, glucose and NEFA to calculate a surrogate marker for IS (RQUICKI). Adiponectin was measured in serum using an in-house developed ELISA. Derivates of reactive oxygen metabolites (dROM) were indirectly measured in serum using a photometric method with N,N,diethyl-1,4-phenylendiamine as chromogen. Every 8 wk, scAT from tailhead was biopsied and snap frozen for genomic DNA isolation. The number of mtDNA copies/cell was measured by multiplex qPCR. Data (mean ± SEM) were analyzed using repeated measures ANOVA and Spearman correlations (SPSS). Decreasing insulin sensitivity throughout the experiment, indicated by decreasing RQUICKI values (P = 0.001), were negatively correlated with dROM (r = -0.543, P = 0.007) and mtDNA copies (r = -0.568, P = 0.005). Moreover, adiponectin concentrations were decreased throughout the experiment (P < 0.05) and tended to be negatively correlated (r = -0.381, P = 0.067) with mtDNA copies. Increased oxidative stress seems to enhance insulin resistance. However, dROM was not associated with serum adiponectin which is known for its insulin sensitizing and lipolysis inhibiting effects. In contrast to reports about insulin resistance being related to reduced mitochondrial content in humans, increasing mtDNA copies in the present study seem to compensate mitochondrial damage caused by enhanced dROM.

Key Words: insulin resistance, oxidative stress, mtDNA

1440 (W200) Serum apelin concentrations in dairy cows receiving different amounts of concentrate and a nicotinic acid supplement. M. Weber[1], L. Locher[2], K. Huber[3], J. Rehage[2], R. Tienken[4], U. Meyer[4], S. Dänicke[4], U. Müller[5], H. Sauerwein[*5], and M. Mielenz[6], *[1]Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Germany, [2]University for Veterinary Medicine Foundation, Hannover, Germany, [3]University of Hannover, Germany, [4]Institute of Animal Nutrition, Friedrich Loeffler Institute, Braunschweig, Germany, [5]University of Bonn, Institute of Animal Science, Germany, [6]Leibniz Institute for Farm Animal Biology, Institute of Nutritional Physiology, Dummerstorf, Germany.*

Apelin, a 77 amino acid preproprotein, which is also known as an adipokine, is suggested to play a physiological role in glucose metabolism. It stimulates glucose uptake by adipose tissue in humans and mice, whereas lipolysis in humans is not affected. This might lead to a decreasing lipolysis during the transition period in cattle and may prevent lipid-related disorders. Nicotinic acid (NA), a known antilipolytic agent, might decrease plasma NEFA concentrations and enhances the response to insulin. As plasma apelin concentrations are decreased by a hypochaloric diet, we hypothesized, that different levels of concentrate in the diet combined with NA supplementation would affect the serum apelin concentrations in dairy cows. Thus the objectives of the present study were to quantify apelin in bovine serum samples and to examine the impact of different levels of concentrate in combination with a NA supplementation on apelin serum concentrations. Serum samples were obtained from 20 pluriparous Holstein-Friesian cows at d −42, −14, 1, 7, 14, 21, 42 and d 100 relative to calving. Until d −42 all cows were fed the same silage-based diet. Between d −42 and d −1 10 animals each were assigned to either a high-concentrate (HC, 60:40 concentrate:roughage) or a low concentrate group (LC, 30:70 concentrate:roughage). Both groups were further subdivided into a control and a niacin group (n = 5), the latter receiving 24 g/d NA (Lonza, Basel, Switzerland) until d 24. Serum apelin concentrations were measured using a commercially available ELISA kit (*P*hoenix Pharmaceuticals, Burlingame, CA) validated for bovine samples. Statistical analysis was done using Mixed-Model procedure followed by Bonferroni correction (SPSS 22); d −42 values were considered as covariate. The serum apelin concentrations were not affected by treatment and time (P > 0.05) and remained on a constant concentration (mean 1.21 ± 0.08 ng/ml). The results of this study indicate that serum apelin concentrations are independent of the prepartum feeding regimen as well as of the stage of lactation.

Key Words: apelin, nicotinic acid, dairy cow

1441 (W201) Nuclear related factor-E2 is downregulated by hyperinsulinemic euglycemia in dairy cows.

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At the onset of lactation the liver undergoes a high load to provide metabolites for milk synthesis in dairy cows. The endocrine and metabolic changes induce inflammation that impairs liver function. The liver is protected by nuclear factor E2-related factor 2 (Nrf2), which is activated by inflammatory signals such as reactive oxygen species (ROS), and has anti-oxidative effects, diminishes inflammatory damage, neutralizes ROS, and suppresses pro-inflammatory signaling. We have studied hepatic mRNA abundance of Nrf2 as a response to long-term (48 h) insulin and β-hydroxybutyrate (BHBA) infusion in mid-lactation dairy cows. Twenty four Holstein dairy cows were randomly assigned to four intravenous treatment groups including an hyperinsulinemic clamp infusion to decrease plasma glucose concentration to 2.5 ± 0.1 mmol/L (HypoG, n = 5), a hyperinsulinemic euglycemic clamp to maintain plasma glucose concentration at pre-infusion level (EUG, n = 6), a BHBA infusion (HyperB, n = 5), and a 0.9% NaCl infusion (Control, n = 8). Liver tissue samples were taken 1 wk before and 48 h after the start of infusion. Changes of hepatic mRNA abundance (RT-qPCR) of several acute-phase proteins and of Nrf2 between before and after 48 h infusions were evaluated by analysis of variance with treatment as fixed effect. SAA and Hp mRNA was up-regulated in all treatment groups ($P < 0.05$) during 48 h infusions. The mRNA of glutathione peroxidase 3 (GPX3), metallothionein (MT) 1A, MT1E, and MT2A was up-regulated after 48 h of infusions in Control ($P < 0.05$). Insulin infusion downregulated mRNA abundance of microsomal glutathione S-transferase 3 (MGST3), MT1E, MT2A, NAD (P) H dehydrogenase, quinone 1 (NQO1), and superoxide dismutase 1 (SOD1) ($P < 0.05$). Changes of GPX3, MGST3, MT1A, MT1E, MT2A, and SOD1 mRNA abundance during 48 h of infusion differed significantly between EUG and Control ($P < 0.05$). Change of mRNA abundance of NQO1 after 48 h of infusions differed significantly between EUG and HyperB ($P < 0.05$). Results show that infusions and experimental condition up-regulated mRNA abundance of APP in all treatments, and up-regulated some of Nrf2 in control, whereas induced hyperinsulinemic euglycemic clamp downregulated most of Nrf2 factors in EUG. It seems that the up-regulation of these factors in Control occurs despite unchanged metabolites during the infusion. Although insulin has an anti-inflammatory role different results observed in both HypoG and EUG. It can be assumed that downregulation of Nrf2 mRNA factors in EUG is related to a decrease of hepatic gluconeogenesis through the decline in glucagon secretion.

Key Words: liver, immune response, cow

1442 (W202) Bovine oocytes in vitro matured in the presence of antioxidants: Implications for intracellular levels of glutathione and reactive oxygen species and blastocyst development.

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Production of reactive oxygen species (ROS) is a physiological process that occurs mainly on mitochondrial metabolism. Some, in vitro culture conditions can lead an increase in ROS production, making the oocytes more susceptible to oxidative stress damage. This study was conducted with the main objective to assess the effects of supplementation of in vitro maturation (IVM) medium with intracellular (cysteine and cysteamine) and extracellular (catalase) antioxidants on the intracellular levels of glutathione (GSH) and ROS in bovine oocytes and its implications on the subsequently embryonic development. *Cumulus*-oocyte complexes were matured in TCM-199 with bicarbonate, hormones and 10% FCS without supplementation (Control group) or supplemented with 0.6 mM cysteine associated with 100 µM cysteamine (C+C group), 100 UI catalase (CAT group) or 0.6 mM cysteine associated with 100 µM cysteamine and 100 UI catalase (C+C+CAT group) for 22 h at 38.5°C in 5% CO_2 in air. A sample of matured and immature oocytes (0 h) were stained (n = 192) with 5 µM of the fluorescent probe 6-carboxy-2'7'-dichlorodihydrofluorescein diacetate (H_2DCFDA; Molecular Probes, Invitrogen, Oakville, Canadá) or stained (n = 246) with ThiolTracker Violet (Glutathione Detection Reagent; Molecular probes, Invitrogen, Oregon) to evaluate ROS and GSH, respectively. Stained oocytes were evaluated immediately under an epifluorescence inverted microscope (excitation 495/510–550 nm and emission 404/526 nm, respectively for H_2DCFDA and ThiolTracker™) and the images were analyzed by Q-Capture Pro image software for determining the fluorescent intensity. Other oocytes were submitted to IVF and the presumptive zygotes were IVC in SOF medium, at 38.5°C in 5% CO_2 in air, for 7 d. The cleavage rates and embryonic development were evaluated, respectively, at 72 and 168 hpi. The differences of fluorescent intensity among groups was compared by ANOVA followed by Tukey's test and embryonic development was analyzed by Chi-square test ($P < 0.05$). The fluorescent intensity for ROS quantification was 1.00 ± 0.12^{a} (0 h), 1.91 ± 0.10^{c} (Control), 1.11 ± 0.04^{a} (C+C), 1.45 ± 0.08^{b} (CAT) and 1.07 ± 0.04^{a} (C+C+CAT). The fluorescent intensity for GSH quantification was 1.00 ± 0.4^{a} (0 h), 0.21 ± 0.01^{bd} (Control), 0.47 ± 0.02^{c} (C+C), 0.32 ± 0.01^{b}

(C+C+CAT) and 0.15 ± 0.01[d] (CAT). The cleavage rates were 73.5[a] (Control), 75.7[a] (C+C), 75.4[a] (CAT) and 73.1[a] (C+C+-CAT). The blastocyst rates were 28.2%[a] (Control), 31.1%[a] (C+C), 33.3%[a] (CAT) and 46.2%[b] (C+C+CAT). In conclusion, supplementation with cysteine, cysteamine and catalase during IVM reduced intracellular ROS levels and improved the embryonic development; however, such improvement was not due to an increase on intracellular amounts of GSH.

Key Words: antioxidants, in vitro maturation, ROS, GSH, blastocyst

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1443 (W203) Heat stress alters adipose adrenergic signaling in lactating dairy cows. G. Xie*[1], L. W. Hall[2], M. Nearing[2], L. C. Cole[2], D. M. Spurlock[3], L. H. Baumgard[3], and R. P. Rhoads[1], *[1]Virginia Tech, Blacksburg, [2]University of Arizona, Tucson, [3]Iowa State University, Ames.*

Malnourished animals mobilize adipose tissue to alleviate the impact of energy deficiency on galactopoiesis, but heat-stressed (HS) lactating cows lessen their dependence on this strategy. One possibility is that lipolytic response becomes refractory to adrenergic signaling. To test this hypothesis, multiparous dairy cows (n = 6; parity = 4 ± 0.9; 436 ± 93 DIM; 721 ± 39 kg BW) housed in climate chambers were fed a TMR consisting primarily of alfalfa hay and steam-flaked corn and subjected to 2 experimental periods: 1) thermoneutral conditions (TN; 18°C, 20% humidity) with ad libitum intake for 9 d and 2) HS conditions (cyclical temperature 31 to 40°C, 20% humidity: min THI = 73, max THI = 86) with ad libitum intake for 9 d. Rectal temperature (Tre) was measured thrice daily at 0600, 1400, and 1800 h. During each period, cows were administered epinephrine intramuscularly (0.02 mg/kg) twice daily from d 6 to 9. Before and after epinephrine treatment, adipose biopsies were obtained from contralateral tailhead areas. Adipose lipolysis and lipogenic-related proteins were measured by western immunoblotting. During period 2, HS caused a 1.3°C increase in Tre compared with TN (P < 0.001). Heat stress reduced DMI by 18% (P < 0.001) and milk yield by 10% (P < 0.01). Epinephrine increased 5' adenosine monophosphate (AMP)-activated protein kinase (AMPK) (P < 0.1), cyclic-AMP response element-binding protein (CREB) (P < 0.05) and hormone sensitive lipase (HSL) (P < 0.05) phosphorylation abundance during TN but not in HS. Beta2 adrenergic receptor (BAR2) abundance was stable in all periods and treatments. Adipose triglyceride lipase (ATGL) protein expression was blunted (P < 0.05) by epinephrine in TN and HS. Fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and phosphorylated ACC protein abundance were decreased (P < 0.05) by HS but did not respond to epinephrine challenge. In contrast, insulin receptor (IR) increased (P < 0.05) in HS regardless of epinephrine administration. Protein kinase B (AKT) phosphorylation tended to increase (P < 0.1) in response to epinephrine during HS. These observations indicate HS may alter adrenergic signaling by blunting lipolytic response in lactating cows. Potential cross talk between epinephrine and insulin may underlie HS adaptation.

Key Words: heat stress, lactation, catecholamine

1444 (W204) Effect of vitamin C supplementation on biochemical parameters and haemagglutination potential of Giant African Land Snail (*Archachatina marginata*) haemolymph. J. A. Abiona*, A. O. Ladokun, J. O. Daramola, D. M. Abioja, E. O. Oke, and O. M. Onagbesan, *Federal University of Agriculture, Abeokuta, Nigeria.*

A study was conducted on the effect of vitamin C on biochemical parameters and haemagglutination potential of Giant African Land Snail's haemolymph (*Archachatina marginata)*. Thirty-two snails with weight range of 100 to 180 g were used for this study. Eight snails were subjected to each of the four treatments which included: concentrate only, concentrate + 50 mg vitamin C, concentrate + 100 mg vitamin C and concentrate + 150 mg vitamin C. After 9 wk, haemolymph was collected from the mantle cavity from all the snails. Parameters monitored were: haemolymph total protein, albumin, globulin, cholesterol, glucose and haemagglutination titre. The result showed that haemolymph albumin, total protein, glucose, and cholesterol were not significantly affected (P > 0.05) by the treatment. However, the different levels of vitamin C with concentrate had significant effect (P < 0.05) on globulin. Snails fed concentrate + 150 mg of vitamin C had the highest least square mean (29.46 ± 1.47). For the haemagglutination test, 100 mg and 150 mg of vitamin C with concentrate for 120 min and 150 min had better haemagglutination titre (strength). It was however recommended that Inclusion of vitamin C in concentrate feed of snails should be encouraged with levels not less than 200 mg/kg of feed.

Key Words: vitamin C, biochemical parameters, haemagglutination potential

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1445 (W205) Effects of grape seed supplementation on blood metabolic profile, immunity and milk production traits of dairy ewes. F. Correddu[1], A. Marzano[1], P. Bonelli[2], P. Nicolussi[2], and A. Nudda*[1], *[1]Dipartimento di Agraria, University of Sassari, Italy, [2]Istituto Zooprofilattico della Sardegna, Sassari, Italy.*

Grapes (*Vitis vinifera*) are rich in polyphenols with approximately 60 to 70% of these being in the seeds. The grape seeds (GS) are rich in proanthocyanidins that exert anti-oxidant and anti-inflammatory activities. The aim of this work was to study the effect of GS by-products in lactating sheep diet on milk production, blood metabolic profile and immune function. Twelve Sarda ewes in the first part of lactation, were divided in two isoproductive groups (1.7 kg/head/d): a control group (CON) and a treated group supplemented with 300 g/d

of grape seed (GS). Milk yield was measured weekly. Blood samples were collected every 2 wk and analyzed for total bilirubin, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), γ glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total protein and urea nitrogen (BUN). Plasma samples were used to perform ELISA to evaluate the anti-OVA IgG titers, CD4 and CD8 cells. Lymphocyte proliferation was determined in vivo in each ewe by the measurement of changes in skin-fold thickness. Milk yield not affected by GS supplementation. All kidney and liver function biomarkers in serum did not differ between dietary groups. A slight suppressing effect of GS on immune activities was evidenced by the reduction of skin-fold thickness, IgG titers, Cd4/Cd8 ratio compared to CON. Grape seed by-product can be supplemented to lactating ewes for 2 mo without altering the immunity and the hepatic and renal metabolism status. *Acknowledgements: Research supported by Cargill–Animal Nutrition Division, Milan, Italy.*

Key Words: grape seeds byproduct, sheep, Immunity

1446 (W206) Determination of glucose metabolism and TCA cycle activity of early antral and late antral feline cat follicles employing [$^{13}C_6$]glucose and mass spectrometry. J. L. Colvin[1], N. Songsasen[2], C. L. Keefer[1], and B. J. Bequette[*1], *[1]Dep. of Animal and Avian Sciences, University of Maryland, College Park, [2]Center for Species Survival, Smithsonian Conservation Biology Institute, Front Royal, VA.*

Improving in vitro culture systems for follicles and oocytes requires knowledge of their metabolism. Thus, our objectives were to establish glycolytic and TCA cycle activity in feline follicles at two developmental stages, and after 13 d of in vitro culture. Paired feline ovaries from sexually mature cats ($\geq$ 1 yr) were acquired from a clinic. Morphologically healthy early (< 0.5 mm o.d.) and late (> 2 mm o.d.) antral follicles with a visible antrum were isolated. Early (n = 10 per cat, n = 9 cats) and late (n = 1 per cat, n = 9 cats) antral follicles were placed into individual wells with culture media (0.5 mL, DMEM containing glutamine and pyruvate plus a 50:50 mix of unlabeled and [$^{13}C_6$]glucose) and incubated for 24 h (5% CO_2) at 38.5°C. To determine whether in vitro culture of early antral follicles leads to acquisition of a late antral metabolism, a group of early antral follicles (n = 10 per cat, n = 9 cats) were encapsulated in 0.5% alginate hydrogel and cultured individually for 13 d with media containing [$^{13}C_6$]glucose the last 24 h. Following incubation, in vivo derived and in vitro cultured early antral follicles were pooled separately by cat for analysis, while late antral follicles were analyzed individually. Metabolites from follicles were extracted, and ^{13}C-isotopomer enrichments of metabolites determined by gas chromatography-mass spectrometry. The TCA cycle intermediate equilibrium partners alanine (pyruvate) and glutamate (α-ketoglutarate) were monitored for calculation of glycolytic and TCA cycle fluxes. Data were analyzed as a mixed model ANOVA with cat and cat age as blocking factors. A greater proportion of pyruvate flux derived from glucose metabolism in late (56%) compared to early (33%) antral follicles, indicating higher rates of glycolysis by late antral follicles. For both early and late antral follicles, only 2% of acetyl-CoA flux derived from glucose, indicating that TCA cycle oxidative metabolism relies on other substrates. Early antral follicles cultured in vitro for 13 d metabolized glucose and had TCA cycle flux activity similar to that of the early antral follicles cultured for 1 d. Thus, in vitro culture of early antral follicles for 13 d did not result in these follicles acquiring a similar metabolism as late antral follicles. The current research demonstrates a metabolic shift between early and late antral follicles derived in vivo, as well as a limited ability of early antral follicles to acquire a late antral metabolism after in vitro culture for 13 d.

Key Words: feline, follicle, metabolism

1447 (W207) Interrelationships between methods of blood mineral measurement in early postpartum dairy cows. B. M. Sweeney[*1], E. M. Martens[1], K. P. Zanzalari[2], J. C. Lawrence[3], and T. R. Overton[1], *[1]Cornell University, Dep. of Animal Science, Ithaca, NY, [2]Prince Agri Products, Inc., Franklin, IN, [3]IDEXX Laboratories, Inc., Westbrook, ME.*

The objective of this study was to determine the relationship between blood minerals measured by different methods and to determine the relationship between blood total (tCa) and ionized calcium (iCa) measured on samples taken from early postpartum dairy cows, as well as the agreement of tCa and iCa for diagnosis of subclinical hypocalcemia (SCH). Seventeen multiparous Holstein dairy cows were sampled 2× in the 24 h period postpartum and 1×/d for the following 4 d. Whole blood was analyzed immediately after collection for iCa by an iSTAT Portable Clinical Analyzer (*P*CA), and serum was analyzed for tCa, Mg and P using both the IDEXX VetTest (VT) and colorimetric methods at a veterinary diagnostic laboratory (DL). Serum total minerals measured by VT vs. DL were highly correlated (tCa $r = 0.95$,$P < 0.0001$;Mg $r = 0.91$,$P < 0.0001$;P $r = 0.97$,$P < 0.0001$). A VT tCa cutpoint with the highest combined sensitivity (96%) and specificity (85%) for diagnosing SCH (defined as DL tCa $\leq$ 8.0 mg/dL) was found to be 8.9 mg/dL as determined by receiver operator characteristic (ROC) analysis. The correlation between tCa measured by DL and iCa measured by PCA was high ($r = 0.89$,$P < 0.0001$). Generally when iCa is used to diagnose SCH, a cutpoint of 4.0 mg/dL iCa is used based on the assumption that iCa constitutes 50% of tCa. Using this assumption, agreement (as determined by McNemar's Test) for diagnosis of SCH ($\leq$ 8.0 mg/dL tCa, $\leq$ 4.0 mg/dL iCa) was poor (Exact $P = 0.06$; Kappa = 0.45, $P < 0.05$). Based on the two samples taken postpartum [7(± 4) h and 20(± 4) h postpartum], this data showed that iCa constituted 58% of tCa in the 24 h postpartum. The iCa cutpoint

with the greatest combined sensitivity (91%) and specificity (87%) for diagnosing SCH was determined by ROC analysis and found to be 4.68 mg/dL. Overall, serum minerals (tCa, Mg and P) measured by standard laboratory techniques are highly correlated with minerals measured by the VT and different cut-points can be used to accurately diagnose SCH with tCa measured by the VT. The relationship between iCa and tCa in the period immediately postpartum must be better characterized before iCa can be used for diagnosis of SCH.

Key Words: ionized calcium, subclinical hypocalcemia, serum minerals

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1448 (W208) Development of a multiplex assay for simultaneous quantification of endocrine analytes.

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Since the advent of the radioimmunoassay in the 1950s, numerous immunologically-based methods have evolved for sample analysis. Although each immunological method possesses unique assets and liabilities, all share limited abilities in range of detection and the number of analytes resolvable simultaneously; most procedures are limited to one analyte determined per replicate per sample. The objective of this study was to adapt technology evolving from the genomics revolution for multiplexed hormonal analysis in livestock. We sought to complete this objective using sequencing technologies and tools: Illumina BeadXpress, Luminex xMAP, and quantitative immuno-PCR. The Illumina BeadXpress and Luminex xMAP both share similar characteristics; each platform consists of a laser spectrum analyzer and a bead-set. Each bead-set contains microscopic beads; each set with unique identifying signatures. As a test of proof of concept, the surface of a bead set was conjugated to an LH antibody. Using these technologies, we were able to establish an assay for LH on the Luminex platform, but not on the Illumina platform. The proprietary nature of both the Luminex and Illumina platforms however, greatly limited assay flexibility. Therefore, we chose to establish an assay for LH using quantitative immuno-PCR. Quantitative immuno-PCR exploits PCR amplification with antibody detection. Briefly, a sandwich immunoassay is performed with capture antibody immobilized to a PCR plate. A second detection antibody is conjugated to an oligonucleotide and after a series of washes, the plate is subjected to quantitative PCR (qPCR). Detection of LH was achieved, but background binding was a problem. Subsequently, to simplify the design and demonstrate proof of concept, a biotinylated oligonucleotide was incubated with streptavidin coated PCR plates and subjected to qPCR. The results of this latter experiment suggested that the assay performed well with over six orders of magnitude linearity. In conclusion, background binding was found to be a major problem with quantitative immuno-PCR but one that is believed to be resolvable. Moreover, our observations suggest that immuno-PCR has the potential to improve detection capabilities of hormonal assays with six orders of magnitude sensitivity, reproducibility, and ultimately in a multiplex capacity with the oligonucleotide serving as both a label and as a barcode for identifying the analyte. The technological leap in capabilities provided by successful multiplexing can be used for understanding the complex interaction of endocrine and metabolic signals in the dynamically changing animal.

Key Words: multiplexing, endocrine profiling, immuno-PCR

1449 (W209) Effect of periconceptual growth hormone injection on feed intake and early fetal development in ewes.

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Researchers have reported improved birth weight and numbers of lambs from ewes treated with growth hormone (GH) at the time of breeding. Therefore, our hypothesis was that the administration of GH at time of breeding could increase DMI which consequently could result in greater early development of the fetus. Thirteen individually-penned ewes (mean BW = 69.8 ± 3.14 kg and BCS = 3.2 ± 0.13 [1-to-5 scale]) were fed a pelleted diet (CP 12.88%, ash 6.48%, fat 1.69%, NDF 37.86%, ADF 23.83% of DM) for ad libitum intake. Estrus was synchronized by administering 2 doses of prostaglandin $F_{2\alpha}$(*P*GF) 11 d apart. At the second dose of PGF, six ewes were injected IM with GH (500 mg) and seven ewes with saline solution (control). The ewes in both treatments were exposed to the same ram at 0700 and 1900 h and allowed to breed. Doppler ultrasound measurements were taken on d 25, 30, 40, and 50 of gestation for fetal length, fetal width, kidney length, kidney width, placentome size, biparietal distance and umbilical blood flow (BF; at d 50 of gestation). Data were analyzed in PROC MIXED (SAS; 2011) to test for the effects of treatment, day and treatment × day. No differences between treatments were observed for BW ($P = 0.16$), BCS ($P = 0.54$) and DMI ($P = 0.84$) after injection of GH. There was a day effect ($P < 0.05$) for fetal length, fetal width, kidney length, placentome size, and biparietal distance with all increasing as gestation advanced. No difference ($P > 0.05$) was observed between treatments for any ultrasound measurements. Growth hormone administration did not influence DMI or conceptus development as measured using ultrasonography. It is still unknown how periconceptual GH treatment could enhance growth and

development of the conceptus, or umbilical BF, after d 50 of gestation when exponential growth of the fetus occurs.

Key Words: ewe, growth hormone, ultrasound

1450 (W210) Relationship between plasma concentrations of thyroid hormones and physiological state of beef cow/calf pairs.

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Triiodothyronine (T_3) and thyroxine (T_4) are important mediators of energy expenditure, growth, and thermogenesis. The prohormone T_4 is converted to the biologically active form, T_3. Relationships between concentrations of T_3 and T_4 in cows and their offspring have not been defined. Spring calving, Angus cow/calf pairs (n = 27) were used to evaluate the relationship between plasma concentrations of T_3, T_4, and T_4:T_3 in cattle of different physiological ages. Calves were weighed at birth and bulls were castrated by banding. Weights of cows and calves were recorded at 48 and 97 d post partum and blood samples were collected at 97 d postpartum. Plasma concentrations of T_3 and T_4 were quantified by RIA. Triiodothyronine, T_4, and the ratio of T_4 to T_3 (T_4: T_3) were analyzed with PROC CORR and PROC MIXED (SAS Inst. Inc.). Plasma concentrations of T_4 did not differ between cows (P = 0.17, 40.5 ± 7.4 ng/ml) and calves (59.2 ± 7.4 ng/ml). Concentrations of T_4 in cows and their calves were not correlated (P = 0.40). Concentrations of T_3 tended to be greater in calves (P = 0.06; 1.99 ± 0.17 ng/ml) compared with cows (0.88 ± 0.17 ng/ml). Concentrations of T_3 in plasma were correlated (r = -0.43, P = 0.03) between cows and their calves. Plasma concentrations of T_4:T_3 in cows were greater (P < 0.001; 49.0 ± 9.3) compared with calves (31.3 ± 9.3) and T_4:T_3 was not correlated between cows and their calves (P = 0.83). The T_4: T_3 in calves was correlated with T_3 (r = 0.45, P = 0.02) in their dams and tended to be positively correlated with T_4 (r = 0.38, P = 0.06) in their dams. Concentrations of T_4 in calves and T_3 in cows were not correlated (P = 0.87). Average daily gain of calves was not correlated with T_4, T_3, or T_4: T_3 in cows or calves ($P \geq 0.12$). Concentrations of T_3 tended to be greater in calves compared with their dams; however, concentrations of T_4 were similar. These results indicate thyroid function in cows and their calves was related as plasma concentrations of T_3 were negatively correlated. Production efficiency of beef cows and calves may be enhanced by identifying individuals with greater metabolic efficiency.

Key Words: thyriod hormone, beef cattle, metabolism

1451 (W211) Follicle-stimulating hormone converges with canonical WNT signaling to enhance Cyp19a1 promoter activity in granulosa cells.

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Biosynthesis of estradiol in the adult ovary requires activation of the tissue specific cytochrome P450 aromatase (*Cyp19a1*) type II promoter (*PII*) by FSH. Canonical wingless-type mammary tumor virus integration-site (WNT) signaling has been recognized to contribute to ovarian regulation of steroidogenesis by increasing the transcriptional co-factor, β-catenin. Recent data suggest WNT3A is inhibitory on FSH mediated mRNA induction of key steroidogenic enzymes and steroid biosynthesis; however, the mechanism by which WNT3A negatively regulates FSH remains to be determined. Therefore, the objective of this study was to investigate the inhibitory effects of WNT3A on FSH-mediated *Cyp19a1* activity. Immunofluorescence was performed on primary rat granulosa cells treated with WNT3A (500 ng/mL) in the presence or absence of FSH (100 ng/mL) for 24 h (n = 4) to determine if FSH prevents WNT3A translocation of β-catenin. Treatment with WNT3A and WNT3A+FSH resulted in nuclear accumulation of β-catenin, while FSH treated cells resembled control groups with the majority of β-catenin remaining at cell membrane. To identify if WNT3A+FSH prevents β-catenin ability to bind the *Cyp19a1 PII*, a 516 bp fragment of the *Cyp19a1 PII* (516-*Cyp19a1 PII*) was transfected into primary cultures of rat granulosa cells, treated with vehicle or WNT3A (500 ng/mL) for 24 h, then co-cultured with or without FSH (100 ng/mL) for an additional 24 h (n = 4). Promoter activity was measured by the luciferase reporter assay and statistical differences for treatment interaction were quantified using one-way ANOVA procedure of SAS. Activity of *Cyp19a1 PII* with WNT3A alone was similar to controls, while FSH treatment increased (P = 0.01) *Cyp19a1 PII* activity 6.65-fold when compared to controls. Co-incubation of FSH and WNT3A was synergistic resulting in a 16.09-fold increase in *Cyp19a1 PII* activity (P = 0.01; n = 4). To evaluate if regions upstream of the 516 bp *Cyp19a1 PII* fragment are responsible for the inhibition of estradiol biosynthesis, the full length *Cyp19a1 PII* (full-*Cyp19a1 PII*) and 2000 bp (2000-*Cyp19a1 Promoter*) upstream of the ATG site on *Cyp19a1* was cloned into a luciferase reporter. Preliminary data suggest *full-Cyp19a1 PII* and *2,000-Cyp19a1 promoter*activity is not synergistic (n = 2) with co-incubation of WNT3A+FSH. Future studies are needed to determine the regions on the promoter responsible for WNT3A inhibition on FSH signaling.

Key Words: Cyp19a1, granulosa cells, WNT

1452 (W212) Effects of various doses of gonadotropin stimulation on reproductive performance of seasonally anestrous ewes. S. L. Rastle-Simpson*, K. N. D'Souza, A. K. Redhead, C. D. Paul, E. N. Keller, and M. Knights, *West Virginia Univeristy, Morgantown.*

The use of exogenous gonadotropins as part of an estrous induction protocol can have beneficial effects on fertility in ewes bred out-of-season. Few studies have evaluated the ability of a mixture of eCG (FSH-like) and hCG (LH-like) (*P*.G. 600, Intervet, Millsboro, DE) to increase fertility in ewes bred out-of-season, specifically following the pre-treatment with progesterone delivered via CIDR devices. Previously, our lab found that administering 3 mL of the gonadotropin mixture 1 d before CIDR removal increased conception rate, pregnancy to first service, lambing rate and the overall percentage of ewes that lambed. The objective of this study was to evaluate the dose effects of a gonadotropin mixture on reproductive performance of seasonally anestrous ewes. Crossbred ewes (n = 200) from three farms in West Virginia and Pennsylvania received CIDR inserts (between the months of May and July) 5 d before introduction of rams. At insert removal, all ewes were assigned randomly to receive a 5-mL injection (i.m., 400 IU eCG, 200 IU hCG) of P.G. 600, a 3-mL injection (i.m., 240 IU eCG, 120 IU hCG) of P.G. 600, or receive no further treatment. The reproductive performance parameters that were measured include pregnancy rate, prolificacy, pregnancy retention, and lambing rate. Analysis of variance was conducted using the GLM procedures of SAS, and least squares means were computed. None of measured reproductive performance parameters was significantly affected by the different doses of gonadotropin stimulation ($P > 0.05$). It is possible that no effect of the gonadotropin stimulation may be due to the high reproductive performance observed in the control ewes. In conclusion, administration of various doses of a gonadotropin mixture at progesterone withdrawal had no effect on reproductive performance of ewes bred outside their normal breeding season.

Key Words: gonadotropin stimulation, anestrus, ewe

1453 (W213) Effect of methionine supplementation on methylation and lipid accumulation of the preimplantation embryo in dairy cows. D. A. Velasco Acosta*[1,2], A. C. Denicol[3], C. S. Skenandore[1], Z. Zhou[1], M. Nunes Corrêa[2], D. N. Luchini[4], P. J. Hansen[3], J. J. Loor[1], and F. C. Cardoso[1], *[1]University of Illinois, Urbana, [2]Federal University of Pelotas, Brazil, [3]Dep. of Animal Sciences, University of Florida, Gainesville, [4]Adisseo S.A.S., Alpharetta, GA.*

The lipid profile of oocytes and early embryo can be influenced by the environment of the cow. Our objective was to determine effect of supplementing rumen-protected methionine on DNA methylation and lipid accumulation in preimplantation embryos of dairy cows. Lactating Holsteins entering their second or greater lactation were randomly assigned to two treatments from 30 ± 2 DIM to 72 ± 2 DIM; Control (CON; n = 5, fed a basal diet with a 3.4:1 Lys:Met) and Methionine (MET; n = 5, fed the basal diet plus Smartamine M to a 2.9:1 Lys:Met). On d 60, dominant follicles greater than 5 mm were aspirated using an ultrasound-guided transvaginal approach. A CIDR device was inserted in all cows after follicular aspiration (d 60) and superovulation began at d 61.5 using FSH treatment equivalent to 400 mg of NIHFSH-P1 (Folltropin) in 8 decreasing doses at 12-h intervals over a 4-d period. During the superovulatory period, all cows received two PGF2α injections at d 63 and 64 (concomitant with the fifth and seventh FSH injections), and CIDR was withdrawn at d 65. Twenty-four h after CIDR withdrawal, ovulation was induced with GnRH. Cows were artificially inseminated at 12 and 24 h after GnRH using a high-fertility sire. Embryos were flushed 6.5 d after artificial insemination. Embryos with stage of development 4 or greater were used for analysis. Methylation was assessed by immunofluorescent labeling with anti-5-methylcytosine while lipid accumulation was assessed by staining with Nile Red. ImageJ software was used for image analysis to determine intensity of labeling. For methylation, fluorescence intensity of nuclear 5-methylcytosine labeling was expressed as a ratio of labeling for DNA using propidium iodide. For lipids, fluorescence intensity of Nile Red staining was compared against a negative control embryo (subtraction of background). Nuclear staining (propidium iodide or Hoescht 33342) was used to count the total number of cells/embryo. A total of 37 embryos were harvested from cows (MET = 16; CON = 21). Statistical analysis was performed using the PROC MIXED of SAS. Cows receiving MET (1661) had greater ($P = 0.021$) lipid accumulation when compared with cows receiving CON (1033). There were no treatment effects ($P > 0.511$) on number of cells or stage of development. In conclusion, cows supplemented with methionine produced embryos with higher lipid concentration when compared to CON which could potentially serve as an important source of energy for the early embryo.

Key Words: methionine, embryo, methylation, lipid

1454 (W214) Expression of Foxp3 in peripheral blood mononuclear cells of pregnant cows. M. A. Paibomesai*[1], R. Machado Ferreira Saran[2], R. da Silva Nunes Barreto[2], F. V. Meirelles[2], and L. J. Oliveira[3], *[1]University of Guelph, ON, Canada, [2]Universidade de São Paulo, Pirassununga, Brazil, [3]Michigan State University, East Lansing.*

Pregnancy has been shown to have great influence over the immune system through the modulation of immune cell types, dampening of the immune response, and display of overall immunodysfunction. It is a period through which the maternal immune response must tolerate long periods of exposure to

foreign antigens produced by the growing fetus throughout gestation. To prevent loss of the fetus the maternal immune system will downregulate specific receptors, such as MHC, and push the system towards to tolerogenic state, as shown in mouse and woman studies. One of these strategies includes the increase in T-regulatory cells, which express the transcription factor FoxP3 and downregulate the immune response after activation. It has been previously shown in mice and woman that as gestation time lengthens the presence of T-regulatory cells increases both locally and systemically. However, this increase in T-regulatory cells has not been well defined in bovine pregnancy. The objective of this study is to determine the expression of FoxP3 transcription factor in $CD4^+$ T cells isolated from pregnant cows (n = 5) and non-pregnant (n = 5) at Day 30 after AI; periparturient (n = 5; 2 to 4 d before parturition) and nonpregnant (n = 5) n lactating cows. $CD4^+$ T cells were isolated by magnetic sorting from selected cows and snap-frozen for RNA extraction and reserve transcription. Gene expression of FoxP3 and PXT-3 were determined by quantitative RT-PCR. Preliminary results show that there is a tendency (P = 0.12) for decreased expression of FoxP3 on pregnant cows at d 30 compared to cow close to parturition. The absence of difference in expression of FoxP3 between pregnant and non-pregnant cows could be due to sample time as well as small group sizes. Additionally, PTX-3 expression is downregulated (P = 0.34) in the periparturient cows, although this was not significant. The decreased expression of PTX3 confirms that peripartum period represents a period of overall immunodysfunction as compared to early gestation. Overall, this study has the potential to identify cows that have higher conception rates due to an immunological system geared towards fetus tolerance.

Key Words: pregnancy, regulatory immune responses, dairy cows

1455 (W215) Luteinizing hormone (LH) profiles after either porcine LH or GnRH treatment in Holstein cows with or without FSH-stimulation.

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Using porcine LH (*P*LH) in lieu of GnRH for synchronizing ovulation in non-stimulated dairy cows resulted in a prolonged and elevated LH profile which favourably altered the expression of intrafollicular proteins associated with improved oocyte competence, and increased pregnancy rates. The wide variability in superovulatory responses and embryo yield in FSH-stimulated cows could potentially be reduced using pLH if the altered (i.e., prolonged and elevated) LH profile attained in non-stimulated cows could be established in superovulated cows. As a first step, our objective was to characterize LH profiles after giving pLH or GnRH in non-lactating Holstein cows subjected to different levels of ovarian stimulation. Cows (n = 13) assigned to no ovarian stimulation (NS; 0 mg FSH) received 100 μg GnRH followed by 500 μg cloprostenol (*P*GF) 7 d later. In ovarian-stimulated groups, cows received decreasing doses of FSH, twice daily over 4-d, with PGF treatments given with the sixth and seventh FSH, to attain either partial stimulation (PS; n = 8, 200 mg FSH) or full stimulation (FS; n = 12, 400 mg FSH). Cows received either 100 μg GnRH or 25 mg pLH 2 d after PGF in NS and 1 d after last FSH in PS and FS groups. Blood samples were collected to determine LH concentrations from 30 min before GnRH- or pLH- treatment, up to 20 h post-treatment and plasma LH concentrations were measured by radioimmunoassay using an anti-bovine LH monoclonal antibody. In GnRH-treated cows, mean (± SE) plasma LH (ng/mL) increased from 0.3 ± 0.1 to a peak of 14.3 ± 1.3 (NS), 6.3 ± 0.8 (PS) and 17.0 ± 2.6 (FS) by 1.5 h, remained elevated for up to 4 h after GnRH treatment ($P \leq 0.01$) returning to baseline by 8 h after treatment in all three groups. In pLH-treated NS and FS cows, plasma LH increased from 0.2 ± 0.2 to a mean peak of 2.1 ± 0.2 and 1.1 ± 0.1 by 1.5 h, and maintained above-basal concentrations ($P < 0.0001$) up to 20 h after treatment, respectively. In pLH-treated PS cows, however, LH concentrations increased from 0.1 ± 0.2 to a mean peak of 1.3 ± 0.2 ng/ml by 3 h, and remained above basal concentrations for up to 10 h post-treatment (0.5 ± 0.2; $P < 0.01$). In summary, LH concentrations in non- and FSH-stimulated cows given pLH remained elevated for a longer period than in cows given GnRH. Whether giving pLH to superovulated cows will reduce variability in ovarian response and improve embryo quality remains to be seen.

Key Words: LH, porcine LH, GnRH, dairy cow