

Physiology and Endocrinology III

W215 Comparison of serum progesterone concentrations from new and used CIDR in Holstein heifers. J. T. Whitley* and C. S. Whisnant, *North Carolina State University, Raleigh.*

The aim of the present study was to determine the progesterone release and concentrations of used Eazi-Breed CIDR (CIDR) devices when compared with new/unused CIDR's. Although used CIDR have been utilized in synchronization protocols data comparing progesterone (P4) concentrations are limited. Pubertal Holstein heifers (13–14 mo of age, 375 ± 11.5 kg BW) were used and randomly assigned to receive either a new (n = 7) or used CIDR (n = 7). A used CIDR in this study was one that was previously removed in a cow for a period of 7 d, removed, washed, and stored. Washing was done by first rinsing with water followed by rinsing with Nolvasan and being allowed to air dry. Storage was in a climate controlled room in a closed drawer. CIDR was inserted 24 h after treatment with prostaglandin F2 α to induce luteolysis. Blood samples were taken by tail vein puncture at 4 times: just before the CIDR was inserted, 1 h later, 24 h later and finally when the CIDR was removed at the end of 7 d. Blood samples were placed on ice for transport and centrifuged, with serum stored at -20°C until assayed for P4 concentrations using a commercially available RIA validated in our laboratory for use with bovine serum. Data were analyzed using PROC GLM of SAS 9.2 for type of CIDR (new vs. used), time and the time by type of CIDR interaction. Serum P4 concentrations (ng/mL) were not different between types of CIDR (Table 1). Serum P4 concentrations increased ($P \leq 0.01$) from time of insertion to 1 h later and remained elevated at all other times. Both new and used CIDR provided a rapid and sustained increase in serum P4 concentration in Holstein heifers.

Table 1.

CIDR	Time 0	1h	24h	7d
NEW ^a	0.6 ± 0.2	4.2 ± 0.5	5.3 ± 0.6	5.1 ± 0.5
USED ^a	0.7 ± 0.2	4.6 ± 0.5	4.8 ± 0.4	4.4 ± 0.6

^aP4 concentrations ng/mL.

Key words: progesterone, CIDR, heifer

W216 Correlation between residual feed intake and metabolic parameters of Nellore heifers. R. H. Branco¹, E. Magnani¹, L. T. Egawa¹, T. L. Sobrinho², S. F. M. Bonilha¹, M. E. Z. Mercadante^{*1}, J. N. S. G. Cyrillo¹, and L. A. Figueiredo¹, ¹Instituto de Zootecnia, Sertãozinho, São Paulo, Brasil, ²Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, São Paulo, Brasil.

Selection against residual feed intake (RFI) has the potential to improve feed efficiency without affecting growth performance or body size, but measuring this trait in cattle is costly. Identification of RFI physiological indicators may facilitate early detection of more efficient heifers. The objective of this study was to examine correlations among RFI, growth performance and metabolic parameters. Nellore heifers (n = 32) from Instituto de Zootecnia – Sertãozinho, São Paulo, Brazil, with averages of 364 kg for BW and 502 d for age were evaluated. Animals were classified for RFI: low RFI (\leq mean + 0.5 SD; n = 17) and high RFI (\geq mean + 0.5 SD; n = 15). Creatine phosphokinase, IGF-I, leptin and cortisol were analyzed. Pearson correlations among variables were calculated by CORR procedure of SAS, and significant correlation between RFI and DMI (Table 1) was found. However, RFI

was not correlated with ADG, once the animals classified as more or less efficient show no difference in the gain. Also correlations between RFI and metabolic parameters were not detected. Cortisol was negatively correlated to leptin, suggesting that animals under stress have lower intake, since the increase in serum cortisol levels lead to reductions in leptin, and this related to the regulation of energy metabolism and feeding behavior. In this study, the animals were not subjected to stress. Thus, more research is needed to identify physiological and genetic markers, which can explain the variations in the physiological bases of the RFI.

Table 1. Pearson correlations among metabolic parameters blood and efficiency measures in Nellore heifers

	ADG	RFI	CPK	IGF-I	LEP	CORT
DMI	0.078ns	0.60**	-0.42*	0.079ns	-0.35ns	0.17ns
ADG		0.074ns	0.38ns	0.098ns	-0.057ns	0.33ns
RFI			-0.19ns	-0.027ns	0.026ns	0.068ns
CPK				0.10ns	0.21ns	0.04ns
IGF-I					-0.07ns	0.06ns
LEP						-0.52**

ns: $P > 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$. CPK=creatin phosphokinase; LEP=leptin; CORT=cortisol.

Key words: beef cattle, efficiency, metabolism

W217 Follicular and ovulatory responses following superovulation treatment with rFSH and HMG in dairy cattle. M. Poorhamdollah^{*1}, H. Kohram^{1,2}, and A. Nejati-Javaremi¹, ¹Department of Animal Science, Faculty College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran, ²Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran.

The unpredictability in the FSH/LH ratio of gonadotropin preparations is considered to be a factor causing variability of superovulatory responses. The aim of this study was to investigate the superovulatory responses in terms of follicular and ovulatory responses during a superovulatory treatment with rFSH and HMG together. Five Holstein dairy cows were selected randomly from the university herd. The estrous cycles were synchronized by 2 successive injections of PGF2 α (vetglan, cloprostenol, Aburaihan, Iran) 11 d apart. Treatments for superovulation were initiated between d 9 to 14 of the estrous cycles with 10 successive injections of 4 shots of rFSH (Gonal-F, Follitropin α , Serono, Switzerland) and 6 shots of HMG (human menopausal gonadotropin, Merional, hFSH-hLH, IBSA, Lugano) at 12-h intervals. A single prostaglandin F2 α injected with the 4th injection of HMG. The ovaries of all cows were examined by ultrasonography (B mode; Pimedical, Falco 100, 8 MHz) on d 9 (day of initiation of the superovulatory treatment), 10, 11, 12, 13, 14 (day of estrus following superovulation) for follicular and ovulatory response, and 7 d after estrus for number of CL and number of non-ovulation follicles. All follicles measured at their largest diameter size. The results showed that the mean number of large follicles (≥ 7 mm) at estrus, CL and non-ovulation follicles at 7- days after estrus were 11.2 ± 1.8, 9.4 ± 1.5 and 1.8 ± 0.8, respectively. There was a high positive correlation (84 and 58%) between the number of CL and the mean number of large follicles at estrus and the number of small follicles (≥ 4 mm) at initiation day of

superovulatory treatment (d 9), respectively. In conclusion, there was a high ovulatory response, high positive correlation between number of ovulated follicles at estrus and numbers of CL 7 d after estrus. Also, the homogeneity between follicles following superovulatory treatment when rFSH and HMG are used together for inducing superovulation was high.

Key words: superovulation, Gonadotropin-releasing hormone, HMG

W218 Adipocyte cell turnover in subcutaneous fat of heifers related to adipocyte cell size. D. Germeroth, S. Häussler*, H. Akter, and H. Sauerwein, *University of Bonn, Germany*.

Adipose tissue mass results not only from adipocyte volume, but also from their number, the latter being determined by cell proliferation and cell loss. In humans, adipocytes probably perish with increased size through apoptosis. In obese subjects apoptosis is presumably overcompensated by emergence of new cells. We hypothesized that the rate of apoptosis and cell proliferation in cattle might depend on adipocyte size. Subcutaneous tailhead fat was collected from 12 non-lactating, non-pregnant Simmental heifers (mean BCS = 5) and from 25 early lactating (d 1 to 105 in milk) Holstein heifers (mean BCS = 3) from different feeding trials. Deparaffinized sections (12 µm) were used for apoptosis detection (TUNEL method); cell proliferation was characterized on cryosections (14 µm) using Ki67-staining. Bovine lymph nodes (apoptosis) and liver (Ki67) were used as positive and negative controls. The portion of apoptotic and proliferating cells was calculated from the mean number of positive stained cells/mean number of total cells x 100. Parametric (Students t-Test) and non-parametric (Mann-Whitney-U-test and Spearman's Rank correlation) tests were used to evaluate the data (SPSS), differences were considered significant at $P \leq 0.05$. The adipocytes from Simmental were larger ($p \leq 0.001$) than from Holstein heifers with mean areas of $8230 \pm 240 \mu\text{m}^2$ and $5146 \pm 491 \mu\text{m}^2$, respectively. Adipocyte area and apoptotic portion were negatively correlated ($r = -0.614$; $p \leq 0.001$), represented by a higher apoptotic portion for heifers having small adipocyte size ($11.37 \pm 2.21\%$) compared to animals with larger ones ($1.03 \pm 0.59\%$). In addition, low cell proliferation rates were observed in both Simmental ($0.43 \pm 0.44\%$) and Holstein ($0.02 \pm 0.01\%$) heifers. Reduction of adipocyte size as observed in early lactating heifers seems to be accompanied by increased apoptosis in fat cells. This loss of cell number is nowhere near enough compensable by cell proliferation. In conclusion, the rate of apoptosis rather than cell proliferation depends on adipocyte size in cattle.

Key words: apoptosis, cell proliferation, adipocyte size

W219 Effect of short-term supplementation and temporary weaning on follicular liquid composition in first-calved Hereford cows. L. Veloz^{1,2}, M. E. Trobo^{1,2}, C. García Pintos^{1,2}, C. Viñoles², and M. Carriquiry^{*1}, ¹*School of Agronomy, UdelaR, Montevideo, Uruguay*, ²*National Research Institute for Agriculture, Tracuarembó, Uruguay*.

To evaluate the effects of short-term supplementation and temporary weaning on follicular fluid composition, first calved Hereford cows ($n = 32$, 388 ± 7 kg BW and 3.6 ± 0.2 units of BCS, scale 1–8) in anestrus and their calves (120 ± 2 kg BW) were used in a randomized block design with a 2×2 factorial arrangement of supplementation (SUP vs. CON), and temporary weaning (with and without), before initiation of the breeding period (103 ± 1 d postpartum). The supplement (2.5 kg/cow of rice bran, 90.3% DM, 10% CP, 9% EE, 14% NDF) was fed

daily for 23 d and calves were temporarily weaned by applying nose plates for 14 d. Cows were injected with 3 prostaglandin (PG) injections 11 d apart. Thirty-six hours after the last PG injection, cows were castrated and all follicles >5 mm were dissected and follicle fluid was aspirated to determine metabolite and hormone composition. Means from a mixed analyses were considered to differ when $P < 0.05$. Follicle size did not differ among groups and averaged 7.0 ± 0.5 mm. Concentrations of estrogen tended ($P = 0.08$) to increase with suckling restriction (4651.1 vs. 1155.2 ± 1777.3 pmol/L). In contrast, progesterone concentrations tended ($P = 0.10$) to increase with supplementation (102.6 vs. 147.0 ± 18.5 ng/mL). The estrogen/progesterone ratio tended to increase ($P = 0.08$) with suckling restriction (34.1 vs. 10.0 ± 14.3). Glucose concentrations were greater in SUP than CON cows (50.8 vs. 57.6 ± 2.5 mg/dL) and suckling restriction tended ($P = 0.08$) to increase glucose in follicular fluid (56.6 vs. 51.7 ± 2.5 mg/dL). Follicular fluid concentrations of NEFA and cholesterol were not affected by nutritional treatment, suckling restriction, or their interaction. Concentrations of estrogen, glucose, and cholesterol as well as the ratio estrogen/progesterone increased with follicle size. In conclusion, nutrition and/or suckling restriction before initiation of the breeding period, in primiparous beef cows in grazing conditions, affected the microenvironment of follicles which could affect reproductive performance

Key words: nutrition, suckling control, ovary

W220 Estrus quantification of early lactation cow cervix physiology: An economical farm innovation. A. Nikkhah*, M. A. Sirjani, and A. A. Assadzadeh, *University of Zanjan, Zanjan, Iran*.

The objective was to quantify cow cervix morphology during estrus phases. Cervix distinctness, central positioning, motility and mucosal secretions were scored daily on a 5-scale basis during proestrus (PE), standing estrus (SE), diestrus (DE) and metestrus (ME) phases in 4 black-white multiparous Holstein cows (50 ± 14 d in milk, 31 ± 3.6 kg milk yield, 643 ± 66 kg BW, 3.0 ± 0.18 BCS) on multiple occasions ($n = 8$). The design was a split-plot with estrus phases as plots. The cervix was video-recorded using a farm apparatus of 45 cm length and 2.7 cm diameter, with internal electrical settings, external polyvinyl cover, front lights, and terminal wires connected to a laptop computer with an image processing program. The score of 1 represented cervixes with fully distinct, central, stable, and mucosal manifestation, and the score of 5 described fully non-separate, non-central, motile, and non-mucosal cervixes. Data were analyzed as mixed models with fixed effect of estrus phase (plot) plus random effects of 'cow within phase' and residuals. Regression was used to relate changes of rectal temperature (RT) and cervix morphology. Results demonstrated a significant ($P < 0.01$) differential order, for $SE > PE > DE > ME$ of an increased cervix distinctness (1.00, 1.20, 3.10, 3.62), greater central positioning (1.13, 1.50, 3.73, 4.15), greater stability (decreased motility) (1.00, 1.50, 2.58, 4.33), and greater mucosal secretions (1.00, 1.50, 3.88, 4.13), respectively, on SE vs. non-SE. The RT was not different ($P = 0.51$) among ME, DE, PE and SE, respectively (38.66 , 38.33 , 38.58 , and $38.83^\circ\text{C} \pm 0.22$). Minor links were found between RT and cervix morphology on SE vs. non-SE phases ($P > 0.20$), except for cervix central positioning (y) and RT (x) changes during ME vs. SE ($y = 3.34 - 1.9x$) ($P = 0.07$). The innovative method proves to be easily applicable to differentiate cervixes on different estrus phases in dairy cows. Its cost-effectiveness (<200 \$US) encourages further research on monitoring reproductive tract health and physiology.

Key words: estrus, physiology, quantification

W221 Effects of maternal metabolizable protein level in late gestation on circulating amino acid concentrations in the ewe and the fetus. L. A. Lekatz¹, M. L. Van Emon², C. S. Schauer², K. R. Maddock Carlin¹, and K. A. Vonnahme¹, ¹*Center for Nutrition and Pregnancy, Department of Animal Sciences, North Dakota State University, Fargo,* ²*Hettinger Research Extension Center, North Dakota State University, Hettinger.*

We have previously reported that feeding 60% of MP requirements during the last third of gestation decreases fetal weight, without altering placental weight, compared with controls by d 130. We hypothesize this fetal weight reduction is due to a decrease in circulating amino acids. Our objective was to examine the effects of maternal MP level during late gestation on circulating amino acid concentrations in the ewe and fetus. Multiparous ewes (n = 11) carrying singletons were assigned with receive an isocaloric diet with 60% (60, n = 4), 80% (80, n = 4), or 100% (100, n = 3) of MP requirements from d 100 until d 130. At surgery on d 130, blood was collected from the maternal saphenous artery (maternal artery, MA), uterine vein of the pregnant horn (UVP), umbilical vein (UMBV) and umbilical artery (UMBA) and amino acids were analyzed. In the MA, Gly was lower ($P = 0.04$) in the 100 compared with the 80 and 60 ewes (226 vs. 369 and 408 \pm 46.1 nmol/ml). In the UVP, Ser, Gly, and Gln were each lower ($P \leq 0.04$) in the 100 compared with the 80 and 60 ewes (27.3 vs. 38.0 and 40.9 \pm 2.91 nmol/ml, 178 vs. 367 and 417 \pm 60.8 nmol/ml, and 192 vs. 229 and 246 \pm 11.4 nmol/ml for Ser, Gly, and Gln, respectively). In the UMBV, Gly was lower ($P = 0.04$) and Val was greater ($P = 0.04$) in the 100 compared with the 80 and 60 ewes (429 vs. 734 and 648 \pm 74.9 nmol/ml and 323 vs. 235 and 175 \pm 36.4 nmol/ml for Gly and Val, respectively). In the UMBA, Gly was lower ($P = 0.02$) and Leu was greater ($P = 0.03$) in the 100 compared with the 80 and 60 ewes (388 vs. 641 and 595 \pm 56.7 nmol/ml and 219 vs. 132 and 101 \pm 28.0 nmol/ml for Gly and Leu, respectively). Also, Val and Ile were greater ($P \leq 0.05$) in the 100 compared with the 60 ewes (302 vs. 156 \pm 33.3 nmol/ml and 113 vs. 50.0 \pm 16.0 nmol/ml, for Val and Ile, respectively), with the 80 ewes being intermediate (214 \pm 33.3 nmol/ml and 69 \pm 16.0 nmol/ml for Val and Ile, respectively). Overall, it appears that decreasing maternal MP from 100% to 80 or 60% of requirements alters amino acid concentrations in both maternal and fetal circulations.

Key words: amino acids, metabolizable protein, pregnancy

W222 Functional genomics and role of integrin beta 5 in cattle fertility. L. Koenig¹, X. Wang¹, A. Kaya², S. Bridges¹, and E. Memili¹, ¹*Mississippi State University, Mississippi State,* ²*Alta Genetics, Inc., Watertown, WI.*

Fusion of male and female gametes at the fertilization is one of the most important events in mammalian developmental biology. Integrins play multiple roles in fertilization, embryogenesis, and implantation. We recently identified a single nucleotide polymorphism in Integrin Beta 5 (ITGB5) associated with bull fertility. Functional significance of this mutation and roles of ITGB5 in fertilization and early embryogenesis are not known. The objectives of this study were to: 1) identify conserved sequence domains and motifs between bovine ITGB5 protein with mouse, human, dog, and rat ITGB5 proteins, 2) determine expression patterns of *itgb5* transcripts in bovine oocytes and early embryos and analyze the results using one way analyses of variance (ANOVA), and 3) determine ITGB5 protein expression in sperm from bulls of varying fertility. Comparative functional genomics, reverse transcriptase real time PCR, and immunoblotting were used to accomplish our objectives. Our results showed that 1) There is an extraordi-

nary degree of conservation (>90% identity of amino acid sequences) of ITGB5 across the mammals indicating that this protein may serve a very important functional role in many species, 2) Transcripts of ITGB5 were highly expressed in bovine oocytes and early embryos; highest expression was at the 2-cell ($P < 0.05$), and 3) ITGB5 protein was detectable in bull spermatozoa. These results provide molecular evidence that ITGB5 is expressed in bovine gametes and embryos and might play important roles at the onset of mammalian development. The findings will help us better understand early mammalian development and identify molecular biomarkers for fertility.

Key words: cattle, fertility, integrin

W223 Male goat vocalizations stimulate LH secretion and estrous behavior in sexually experienced but not in sexually inexperienced goats. J. A. Delgadillo*, J. Vielma, H. Hernández, J. A. Flores, G. Duarte, I. G. Fernández, and G. Fitz-Rodríguez, *Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, Mexico.*

The objective of the current study was to determine the response of sexually inexperienced and experienced goats to buck vocalizations by measuring their secretion of LH and estrous behavior. Males (n = 3) were rendered sexually active during the non-breeding season by exposure to 2.5 mo of long days (16 h of light by d) from November 1st. From 20 d of age, females were isolated from any auditory, visual and tactile cues from males so that, once in adulthood, they had no sexual experience. By contrast, a group of females had visual, auditory, olfactory and tactile contact with male goats, but copulations were prevented by a fence. On April 4th (d 0; 11:00 h) a stimulus group (n = 5) of anestrus females was exposed to photoperiod treated males in a light proof-building; under these conditions, males produced vocalizations that were reproduced through a microphone-amplifier-loudspeaker system. The anestrus sexually inexperienced and experienced groups (n = 6 each, females had 15 mo of age,) were kept in 2 open pens and exposed during 5 consecutive days to the buck vocalizations coming from the males exposed to stimulus females. LH pulsatility was determined every 15 min from 4 h before to 8 h after introducing the males. Behaviors were recorded twice daily. The LH pulses frequency was analyzed by a 2-way ANOVA with repeated measurements (group and time). The estrous behavior was compared by the Fisher exact test. The number of LH pulses did not differ between sexually inexperienced and experienced goats before exposition to vocalizations of bucks (0.4 + 0.2 in both groups; $P > 0.05$). In contrast, vocalizations induced an increase of pulses of LH in experienced (1.7 + 0.4) but not in inexperienced females (1.0 + 0.3; $P < 0.05$). The frequency of mounting attempts (50) and the acceptance of mounts (36) were greater in experienced than in inexperienced females (1 and 7, respectively; $P < 0.01$). These results indicate that the lack of sexual experience of females is associated with low endocrine and behavioral responses to buck vocalizations.

Key words: caprine, sexual behavior, endocrine activity

W224 Profiling bioenergetics and metabolic stress in cells derived from commercially important fish species. B. Beck* and A. Fuller, *Stuttgart National Aquaculture Research Center, Stuttgart, AR.*

As organisms intimately associated with their environment, fish are sensitive to numerous environmental insults which can negatively affect their cellular physiology. For our purposes, fish subject to intensive farming practices can experience a host of acute and

chronic stressors such as changes in dissolved oxygen, temperature, and water quality; all of which can result in metabolic perturbations on a cellular level. Thus, in the present study, we sought to further our understanding of cellular metabolism in fish and to examine the stress response of cells derived from commercially relevant fish species (catfish, white bass, fathead minnow). We employed a Seahorse Bioscience XF24 Extracellular Flux (EF) Analyzer, an instrument which detects changes in oxygen (O₂) levels and pH within the media directly surrounding cells. By measuring the O₂ consumption rate (OCR), an indicator of mitochondrial respiration, we determined that all cells tested exhibited a markedly aerobic phenotype (OCR > 100 pMol/min). Simultaneously, we measured the extracellular acidification rate (ECAR), an indicator of glycolysis, and found that in all cell lines tested the ECAR was very low (<5 mpH/min). Next, we performed a mitochondrial function protocol whereby compounds modulating mitochondrial respiration were sequentially exposed to cells (oligomycin→FCCP→rotenone). For each cell type, this assay provided us with basal and maximal OCR, O₂ consumption dedicated to ATP production, O₂ consumption from ion movement across the mitochondrial inner membrane, the reserve respiratory capacity, and O₂ consumption independent of Complex IV of the electron transport chain. From these informative bioenergetic parameters we generated distinct metabolic signatures for each cell type. These findings are the first description of EF technology employed on fish cell lines and provide key proof-of-concept data demonstrating the utility of fish cells as tools for modeling bioenergetics. We hope to extend these findings to develop assays predictive of how fish may cope with cellular insults encountered in production settings.

Key words: extracellular flux, mitochondria, bioenergetics

W225 Conjugated linoleic acid and rosiglitazone attenuate lipopolysaccharide-induced TNF- α production by bovine immune cells. M. C. Perdomo and L. Badinga*, *University of Florida, Gainesville.*

Lipopolysaccharide (LPS) modulates innate immunity through alteration of cytokine production by immune cells. The objective of this study was to examine the effect of exogenous conjugated linoleic acid (CLA) and peroxisome proliferator-activated receptor gamma (PPAR- γ) agonist, rosiglitazone, on LPS-induced tumor necrosis factor α (TNF- α) production by cultured blood from prepubertal Holstein heifers (mean age = 5.5 mo). Compared with unstimulated cells, addition of LPS (10 μ g/mL) to the culture medium increased peripheral blood mononuclear cell (PBMC) proliferation up to 2.5-fold. Co-incubation with interferon gamma (IFN- γ , 5 ng/mL) further stimulated ($P < 0.01$) the proliferative response to LPS. Lipopolysaccharide increased ($P < 0.01$) TNF- α concentration in cultured whole blood in a dose- and time-dependent manner. The greatest TNF- α stimulation occurred after 12 h of exposure to 1 μ g/mL of LPS. Co-incubation with trans-10, cis-12 (t10,c12) CLA isomer (100 μ M) or rosiglitazone (10 μ M), a PPAR- γ agonist, decreased LPS-induced TNF- α production by 13 and 29%, respectively. Linoleic acid (LA) and cis-9, trans-11 (c9,t11) CLA isomer had no detectable effects on LPS-induced TNF- α production. The PPAR- γ agonist-induced TNF- α attenuation was reversed when blood was treated with both rosiglitazone and GW9662, a selective PPAR- γ antagonist. Addition of rosiglitazone to the culture medium tended to reduce NF- κ Bp65 concentration in nuclear extracts isolated from cultured PBMC. Results demonstrate that LPS is a potent inducer of TNF- α production in cultured bovine blood, and that t10,c12 CLA and PPAR- γ agonists attenuate the TNF- α response to LPS in vitro.

Additional studies are needed to fully characterize the involvement of NF- κ B in LPS-signaling in bovine blood cells.

Key words: CLA, TNF- α , cattle

W226 Influence of nitrogen and sulfur intake on bovine uterine pH. J. K. Grant^{*1}, P. Steichen², C. L. Wright¹, K. A. Vonnahme², M. L. Bauer², J. S. Jennings³, and G. A. Perry¹, ¹*Department of Animal and Range Sciences, South Dakota State University, Brookings,* ²*Department of Animal Science, North Dakota State University, Fargo,* ³*Alltech Animal Nutrition, Brookings, SD.*

Previous research has reported that high protein and sulfur intake decreases uterine pH in cattle. Therefore, the objective of this study was to determine the effect of high N and S intake on uterine pH. Holstein and Angus-cross heifers (n = 20; 337.5 \pm 8.4 kg of BW) were randomly assigned to 1 of 4 diets: control (C; 13.4% CP and 0.17% S); high N (N; C plus urea supplement to achieve 18.5% CP); high S (S; C plus calcium sulfate to achieve 0.43% S); or both high N and S (NS). Diets were individually fed at 2.6% of BW using Calan gates and estrus was synchronized to occur on d 12 after the experiment began. Blood samples were collected daily to determine plasma urea nitrogen (PUN), sulfate (d 0, 3, 7, 11, and 15), and progesterone concentrations. Uterine pH was measured on d 15, 19, 23, and 27 (d 3, 7, 11, and 15 of the estrous cycle). There was a treatment, time, and treatment x time interaction ($P < 0.01$) on PUN concentrations. Starting on d 2, PUN concentrations were increased in N and NS, which were not different ($P > 0.05$), compared with C and S ($P < 0.01$), which were not different ($P > 0.05$). There was an effect of treatment ($P < 0.01$) on sulfate concentrations, with concentrations being increased in S compared with C, N, and NS ($P \leq 0.01$), with NS increased compared with C ($P = 0.04$). In addition, sulfate concentrations were increased on d 3 compared with d 7 ($P = 0.04$) and 15 ($P < 0.01$), but there was no treatment x time interaction ($P = 0.81$). There was no effect of treatment ($P = 0.55$) or a treatment x time interaction ($P = 0.16$) on progesterone concentrations, but there was an effect of time ($P < 0.01$), with increasing concentrations after estrus consistent with normal CL formation. Uterine pH was increased in N and NS compared with C ($P < 0.02$), while S was not different from any treatment ($P > 0.11$). There was no effect of time ($P = 0.26$) or treatment x time interaction ($P = 0.71$) on uterine pH. In summary, uterine pH was increased in N and NS compared with C, while S was intermediate, and correlated with increased PUN concentrations.

Key words: nitrogen, sulfur, uterine pH

W227 Influence of sperm fertility-associated antigen status on nulliparous Nelore heifer fertility at first-service timed AI. J. C. Dalton^{*1}, L. Deragon², J. L. M. Vasconcelos³, A. Ahmadzadeh⁴, and R. F.G. Peres⁵, ¹*University of Idaho, Caldwell,* ²*Alta Genetics Brazil, Uberaba, MG, Brazil,* ³*FMVZ-UNESP, Botucatu, SP, Brazil,* ⁴*University of Idaho, Moscow,* ⁵*Agropecuária Fazenda Brazil, Barra do Garças, MT, Brazil.*

The objective was to determine whether the presence of sperm fertility-associated antigen (FAA; a 31 kDa heparin binding protein) can be used to assess potential fertility of sperm for use at first-service timed AI (TAI). Following determination of FAA status by use of a lateral flow cassette, 6 Nelore bulls were selected based on FAA status (FAA-negative: n = 3; FAA-positive: n = 3) and their ability to produce neat semen with characteristics equal to or greater than 70% morphologically normal sperm and 60% estimated progressive motility

before cryopreservation. Ejaculates were collected by artificial vagina and extended semen was cryopreserved in 0.25-mL straws (30×10^6 sperm). Nulliparous Nelore heifers ($n = 617$) at a single location in Mato Grosso, Brazil, were evaluated for body condition score (BCS; 1–5 scale) and enrolled in a first-service TAI program. On d 0 heifers received an intravaginal insert containing 1.9 g progesterone (CIDR) and an injection of estradiol benzoate (2.0 mg i.m.). On d 7, all heifers received an injection of prostaglandin $F_{2\alpha}$ (12.5 mg i.m.). On d 9 CIDR inserts were removed and all heifers received an injection of estradiol cypionate (0.6 mg i.m.) and an injection of eCG (200 IU i.m.). On d 11, all heifers received TAI 48 h after CIDR removal. Fertility, as measured by pregnancy/TAI (P/TAI), was different ($P = 0.04$) between FAA-positive and FAA-negative bulls (33.7% vs. 40.7%, respectively). There was no effect of AI technician or BCS on P/TAI. In this study using a limited number of bulls (FAA-negative: $n = 3$; FAA-positive: $n = 3$) and TAI, it appears that FAA-negative status was not a limiting factor as nulliparous Nelore heifers achieved greater P/TAI with sperm from FAA-negative bulls. These results appear to be contradictory to a previous report of greater fertility following the use of FAA-positive bulls in AI (Sprott et al., *J. Anim. Sci.* 2000:78:795–798).

Key words: sperm, timed AI, fertility

W228 Feeding rumen-protected polyunsaturated fatty acids (PUFA) to high-producing dairy cows: II. Effects on serum concentrations of progesterone and insulin. M. M. Reis¹, R. F. Cooke², B. I. Cappellozza², and J. L. M. Vasconcelos*¹, ¹UNESP – Faculdade de Medicina Veterinária e Zootecnia, Botucatu, SP, Brazil, ²Oregon State University–Eastern Oregon Agricultural Research Center, Burns.

The objective was to determine if the productive and reproductive benefits of rumen-protected PUFA supplementation to high-producing dairy cows are due to increased circulating concentrations of progesterone (P4) and insulin. In this study, 765 primiparous and multiparous lactating Holstein cows, with estimated production of at least 9,000 kg of milk/yr, were randomly allocated approximately 30 d postpartum to 1 of 10 free stalls, where they remained throughout the lactation. Each free stall was assigned randomly to receive a diet, balanced to meet the nutritional requirements of lactating dairy cows, without (control) or with (PF) the inclusion of 250 g/cow daily of a rumen-protected PUFA source (Megalac-E; QGN, Rio de Janeiro, Brazil). Control and PF diets were iso-energetic and iso-nitrogenous, and the PUFA source was offered during the first feeding of the day (0600 h). Between 45 and 60 d postpartum, cows were randomly assigned to fixed-time AI (TAI) or embryo transfer (ET). Blood samples were collected 7 d after TAI or on the day of ET for determination of serum P4 and insulin concentrations. Primiparous PF cows had greater ($P < 0.01$) P4 concentrations compared with control cohorts (4.4 vs. 3.2 ng/mL, respectively), whereas no treatment differences were detected in multiparous cows ($P > 0.61$). Similarly, PF primiparous tended ($P = 0.08$) to have greater insulin concentrations compared with control cohorts (10.5 vs. 7.5 μ IU/mL, respectively), whereas no treatment differences were detected in multiparous cows ($P > 0.43$). When P4 and insulin were analyzed independently of treatments, primiparous cows with insulin concentrations equal or greater than the median (7.35 μ IU/mL) had greater ($P < 0.01$) P4 concentrations compared with primiparous cows with insulin below the median (5.4 vs. 3.3 ng/mL, respectively). The same outcome, however, was not detected among multiparous cows ($P > 0.29$). In summary, rumen-protected PUFA supplementation at 250 g/d increased circulating concentrations of P4 in primiparous lactating cows, which may be attributed to concurrent increases in insulin concentrations.

Key words: PUFA, insulin, progesterone

W229 Feeding rumen-protected polyunsaturated fatty acids (PUFA) to high-producing dairy cows: I. Effects on milk production and reproductive performance. M. M. Reis¹, R. F. Cooke², S. Soriano⁴, F. L. Aragon³, M. B. Veras³, and J. L. M. Vasconcelos*¹, ¹UNESP – Faculdade de Medicina Veterinária e Zootecnia, Botucatu, SP, Brazil, ²Oregon State University–Eastern Oregon Agricultural Research Center, Burns, ³Pioneiros Veterinary Clinic, Carambei, PR, Brazil, ⁴Colorado Dairies, Araras, SP, Brazil.

The objective was to determine if rumen-protected PUFA supplementation benefits performance and reproduction of high-producing dairy cows. In this study, 1,083 primiparous and multiparous lactating Holstein cows, with estimated production of at least 9,000 kg of milk/yr, were randomly allocated approximately 30 d postpartum to 1 of 10 free stalls, where they remained throughout the lactation. Each free stall was assigned randomly to receive a diet, balanced to meet the nutritional requirements of lactating dairy cows, without (control) or with (PF) the inclusion of 250 g/cow/d of a rumen-protected PUFA source (Megalac-E; QGN, Rio de Janeiro, Brazil). Control and PF diets were iso-energetic and iso-nitrogenous, and the PUFA source was offered during the first feeding of the day (0600 h). Milk production and composition were evaluated during the initial 43 wk of lactation. Between 45 and 60 d postpartum, cows were randomly assigned to fixed-time AI (TAI), embryo transfer (ET) or conventional AI. Pregnancy was verified by transrectal ultrasonography 60 d after TAI, ET or AI. Results were analyzed using the PROC MIXED and GLIMMIX of SAS. Cows receiving PF had greater ($P < 0.01$) milk production compared with control (38.7 vs. 36.2 kg/cow/d, respectively). However, PF cows had reduced ($P < 0.01$) milk protein and fat content compared with control cows (3.09 vs. 3.11% of protein and 3.42 vs. 3.55% of fat, respectively). Still, PF cows had greater ($P < 0.01$) fat corrected milk production (3.5% milk fat) compared with control cows (37.3 vs. 36.0 kg/cow/d, respectively). Independently of breeding procedure (TAI, AI, or ET), no treatment effects were detected on first service pregnancy rates (18.7 vs. 20.2% of pregnant cows/total cows for PF and control, respectively; $P = 0.78$) or number of services required for pregnancy (2.23 vs. 2.28 services per pregnancy for PF and control cows, respectively; $P = 0.73$), even when milk production served as covariate. In summary, rumen-protected PUFA supplementation to high-producing dairy cows enhanced milk production without impairing reproductive performance.

Key words: PUFA, milk production, reproduction

W230 Puberty induction in Nelore heifers receiving eCG and/or estradiol cypionate at the end of the estrus synchronization protocol. A. Rodrigues¹, R. Peres*³, A. Lemes², T. Martins¹, F. Aono¹, M. Pereira¹, H. Graff³, E. Carvalho³, and J. L. M. Vasconcelos¹, ¹FMVZ-UNESP, Botucatu, SP, Brazil, ²ESALQ-USP, Piracicaba, SP, Brazil, ³Agropecuária Fazenda Brasil, Barra do Garça, MT, Brazil.

The aim of this study was to evaluate if eCG and/or estradiol cypionate (ECP) administration at the end of an estrus synchronization protocol containing a previously used (4th use) intravaginal progesterone device (CIDR) increases induction of puberty in prepubertal Nelore heifers. Heifers were evaluated for puberty status 7 d before and at CIDR insertion (d 0) via transrectal ovarian ultrasonography and classified as prepubertal if a corpus luteum was not detected. Only prepubertal heifers were utilized in the present study. After CIDR removal (d

12), heifers detected in heat were inseminated according to the Trimmer system for 7 d, whereas 8 d after CIDR removal heifers not detected in estrus were again evaluated for corpus luteum presence. Data were analyzed by logistic regression using PROC LOGISTIC of SAS. In Exp. 1, 896 heifers were used and randomly received 200 IU of eCG (G2) or no treatment (control) at CIDR removal (d 12). Heifers that received G2 had greater ($P < 0.01$) puberty induction and heat detection compared with control (71.9 and 53.3% of puberty induction and 34.3 v. 27.5% heat detection rate, respectively). Conception rates were not affected ($P > 0.10$) by treatments (45.1 vs. 43.5% for G2 and control, respectively). In Exp. 2, 401 heifers randomly received G2, 200 IU of eCG + 0.5 mg ECP (G3), or control at CIDR removal (d 12). Heifers that received G3 had greater ($P = 0.01$) estrus detection and puberty induction compared with G2 and control heifers (56.1, 34.8 and 21.5% estrus detection rate and 88.3, 75.0 and 45.6 puberty induction, respectively). Conception rate were similar ($P > 0.10$) among treatments (46.7, 34.7, and 33.3 for control, G2, and G3, respectively). In Exp 3, heifers randomly receive 0.5 mg of ECP (E1), G2, G3, or control at CIDR removal (d 12). Heifers receiving G3 had greater ($P = 0.02$) puberty induction compared with E1, G2, and control (88.7, 75.3, 82.2, and 57.9%, respectively). In conclusion, the use of ECP, eCG, and particularly eCG + ECP at CIDR removal increased the number of Nelore heifers cycling and detected in heat at the beginning of the breeding season.

Key words: heifers, estradiol and eCG, puberty

W231 Repeated exposure to human chorionic gonadotropin causes development of antibodies in some lactating dairy cows. J. O. Giordano*, M. C. Wiltbank, and P. M. Fricke, *Department of Dairy Science, University of Wisconsin-Madison, Madison.*

Our objective was to determine if repeated exposure of lactating dairy cows to human chorionic gonadotropin (hCG) would induce an antibody (Ab) response against hCG. Cows ($n = 45$) enrolled in a synchronization of ovulation experiment either received an hCG injection (hCG; 2000 IU i.m.) or no treatment (CON) at 18 d after a timed AI. A subgroup of cows ($n = 27$) from the original group received a 2nd hCG injection 35 d after the 1st injection, and another subgroup ($n = 18$) of cows received a 3rd hCG injection 35 d after the 2nd hCG injection. Blood samples were collected at 0, 7, 14, 21, and 28 d after hCG or CON. A binding radioimmunoassay for hCG Ab was used to detect hCG Ab in serum samples. A positive Ab response (6.34% bound) was defined as 3 standard deviations above CON binding. The proportion of cows presenting an Ab response at 0 and 14 d after hCG was compared through one tailed Fisher's exact test, whereas difference in Ab bound at 0, 7, 14, 21, and 28 d was compared with PROC MIXED of SAS. No cows had hCG Ab at Day 0 before 1st hCG. At 14 d after 1st hCG, no difference ($P = 0.48$) was observed between CON (0/22) and hCG (1/20) cows for percentage Ab positive. At 2nd hCG, no difference ($P = 0.59$) was observed on Day 0 between CON (0/11) and hCG (1/16) cows, whereas a tendency ($P = 0.06$) was observed at 14 d [(CON = 0/5) vs. (hCG = 8/16)]. At the 3rd hCG injection no difference ($P = 0.16$) was observed on Day 0 between CON (0/6) and hCG (4/12) cows, whereas a greater ($P = 0.05$) proportion of hCG cows (6/9) had hCG Ab at 14 d vs. CON cows (0/4). Treatment, time, and treatment by time affected ($P < 0.05$) the average % Ab bound after the 2nd and 3rd hCG injection. Cows that received hCG had greater % Ab Bound at 7, 14, 21, and 28 d after hCG, with the greatest binding observed at 14 d. We conclude that some but not all lactating dairy cows developed an Ab response after repeated exposure to hCG and that maximum response is observed within 14 d of hCG injection.

Key words: hCG, immune response, dairy cow

W232 Synchronization of dairy heifers with a modified 5-day CIDR-PGF_{2α}-GnRH timed AI protocol. J. Howard*^{1,2}, K. Carnahan¹, C. Autran¹, J. Branan², R. Kasimanickam³, G. Sasser², and A. Ahmadzadeh¹, ¹University of Idaho, Moscow, ²BioTracking LLC, Moscow, ID, ³Washington State University, Pullman.

It has been shown that the use of a CIDR insert, in conjunction with a GnRH-PGF_{2α} fixed time AI(TAI) protocol improves pregnancy per AI (P/AI). But due to variability in the results of previous studies it is not clear whether the 1st GnRH administration at the time of CIDR insertion in a 5-d CIDR protocol improves P/AI in heifers. The objective of this experiment was to evaluate the effect GnRH at the time of CIDR insertion, in a 5-d CIDR-Cosynch timed artificial insemination (TAI) protocol on P/AI in dairy heifers. Holstein replacement heifers ($n = 234$), received a CIDR on d 0. Subsequently heifers were paired by age and assigned randomly to receive either 100 ug of GnRH (GnRH-CIDR; $n = 117$) or no GnRH treatment (Control; $n = 117$). On d 5, the CIDR was removed and all heifers received 25 mg PGF_{2α} (d 5). On d 8 (72 h after CIDR) all heifers received GnRH and TAI. Estrus activity was monitored using tail chalk methods from d 5 to d 8. Pregnancy was diagnosed by ultrasound and a Pregnancy specific protein B (PSPB) based ELISA (BioPRYN) on d 32 and 45 after AI. Blood samples were collected in a subgroup of heifers ($n = 113$) on d 0 to determine progesterone (P4) concentrations. There was no effect of bull or technician on P/AI on d 32 or d 45. Mean P4 concentrations on d 0 were not different between groups and averaged 3.6 ± 0.5 ng/mL. Pregnancy per AI did not differ between treatments (GnRH CIDR 57.2% vs. Control 61.5%) on d 32 or 45. Only 18% of heifers were not detected in estrus and P/AI was less ($P < 0.05$) in heifers that were not detected in estrus (41.8% vs. 63.3%). The results of this experiment indicate GnRH administration at CIDR insertion, in 5-d CIDR-Cosynch may not have beneficial effects on P/AI in Holstein heifers.

Key words: dairy heifer, timed AI, GnRH

W233 Prepartum 2,4-thiazolidinedione administration increases plasma tumor necrosis factor alpha in transition dairy cows. K. M. Schoenberg*¹, K. L. Perfield², J. K. Farney³, B. J. Bradford³, and T. R. Overton¹, ¹Cornell University, Ithaca, NY, ²Elanco Animal Health, Greenfield, IN, ³Kansas State University, Manhattan.

Thiazolidinediones (TZD) are potent ligands for peroxisome proliferator-activated receptor-gamma (PPAR γ), and TZD administration has been shown to alter lipid metabolism and energy status in transition dairy cows. Tumor necrosis factor α (TNF α) is an inflammatory cytokine which may also play a role in metabolic health during the transition period. The objective of this experiment was to determine the effect of prepartum TZD administration on plasma TNF α and further characterize TNF α throughout the transition period. Holstein cows ($n = 31$) entering their second or greater lactation were administered 0, 2.0, or 4.0 mg TZD/kg BW by intrajugular infusion once daily from 21 d before expected parturition until parturition. Plasma samples were analyzed for TNF α on d -14, -3, -1, 1, 3, 7, 35, and 49 relative to parturition via a recently developed bovine TNF α enzyme-linked immunosorbent assay (ELISA). Results were analyzed with a mixed model including repeated measures over time and a covariate sample collected at d -22. Data transformation was required to meet assumptions of normality for statistical analysis and values reported are back-transformed. Independent of day, plasma TNF α was increased linearly

by increasing TZD dose (2.63, 3.72, 3.95 pg/mL; $P = 0.01$). The temporal pattern (effect of day; $P < 0.01$) for plasma TNF α was such that it lowest (2.85 pg/mL) during the postpartum period (d + 7 to d + 49), highest (4.18 pg/mL) during the prepartum period (d -14) and intermediate (3.32 pg/mL) during the transition period (d -3 to +3). Contrasts of the effect of period showed that prepartum values were significantly different from both the transition period ($P < 0.01$) and the postpartum period ($P < 0.001$). These results suggest that TNF α may be an important metabolic regulator during the transition period. Somewhat surprisingly, TZD administration increased TNF α concentrations independent of day relative to calving. The effects of TZD on TNF α may be confounded in this case with effects on other regulators such as leptin.

Key words: transition cow, thiazolidinedione, tumor necrosis factor alpha

W234 Effect of dietary β -glucan on growth performance, fecal microbial shedding and immunological responses after lipopolysaccharide challenge in weaned pigs. T. X. Zhou*, B. U. Yang, and I. H. Kim, *Dankook University, Cheonan, Choongnam, South Korea.*

Two experiments were conducted to investigate the effects of β -glucan on growing performance and immune function in weaned pigs after LPS challenge. In Exp. 1, 40 weaned pigs (7.89 \pm 0.84 kg of BW and 21 \pm 2 d of age) were used in a 28 d feeding trial. All pigs were randomly allotted to 1 of 2 treatments (0 or 0.1 g/kg of β -glucan in the diet) with 4 replicate pens per treatment and 5 pigs per pen. Dietary β -glucan decreased ($P < 0.05$) the number of *E. coli*. In Exp. 2, a total of 20 weaned barrows (6.69 \pm 0.24 kg of BW and 21 \pm 2 d of age) were used to investigate the immunological response after LPS challenge. Pigs were fed 0 or 0.1 g/kg dietary β -glucan for 42 d. On d 42, pigs (n = 5) in each treatment were injected i.p. with *E. coli* lipopolysaccharide or sterile saline solution at a concentration of 100 μ g/kg of BW. Dietary β -glucan increased leukocytes counts at 2, 4 and 6 h, and lymphocyte concentration at 2, 4 and 6 h, and LPS challenge increased ($P < 0.05$) leukocytes counts at 2, 4, 6, and 8 h and increased ($P < 0.05$) lymphocyte concentration at 2, 4, and 6 h post-challenge and an interaction ($P < 0.05$) was observed. LPS challenge increased the rectal temperature at 2, 4, 6, and 8 h post-challenge. Dietary β -glucan reduced plasma TNF- α concentration while LPS challenge increased ($P < 0.05$) blood TNF- α concentration at 2, 4, and 6 h. Dietary β -glucan increased ($P < 0.05$) the concentration of the cluster of differentiation antigens 4 and 8 (CD4+ and CD8+) cells at 2, 4, 6 h and 4, 6 h post-challenge, respectively, and LPS challenge increased ($P < 0.05$) CD4+ and CD8+ cell concentrations at 2, 4 and 6 h post-challenge; an interaction ($P < 0.05$) between β -glucan and LPS challenge was observed. The CD4+: CD8+ ratio was decreased ($P < 0.05$) by LPS challenge and dietary β -glucan at 2, 4, 6 and 8 h post challenge and an interaction ($P < 0.05$) was observed at 4 and 6 h post challenge. In conclusion, dietary β -glucan can decrease *E. coli* numbers but not affect growth performance in weaned pigs and offer benefits on immune function in weaned pigs challenged with LPS.

Key words: beta-glucan, lipopolysaccharide challenge, pigs

W235 Difference in the expression of components of the GHR/IGF-I axis in follicular granulosa cells and corpus luteum in cows. A. Schneider^{1,2}, L. F. M. Pfeifer¹, M. N. Corrêa¹, and W. R. Butler^{*2}, ¹Universidade Federal de Pelotas, Pelotas, RS, Brazil, ²Cornell University, Ithaca, NY.

The aim of this work was to evaluate the difference in the expression of components of the GHR/IGF-I axis in follicular granulosa cells and corpus luteum (CL) in cows. Expression of IGF-I in granulosa cells is controversial, even though it is easily detected in the CL. Ovaries were collected from cows during slaughter. Follicles (7 estrogen-active follicles [EAF] and 7 atretic follicles [ATF]) were dissected from the stroma, FFL was aspirated and the follicle walls immersed in RNALater. To recover granulosa cells, follicular walls were removed from the RNALater, halved, scraped and washed with cold saline. Granulosa cells were recovered by centrifugation at 2000 x g for 3 min. The CL (n = 7) were also dissected. Total RNA was isolated and real-time PCR used to evaluate suppressor of cytokine signaling (SOCS-1, -2 and -3), GHR, IGF-I, IGF-II and ER α mRNA expression according to the $\Delta\Delta$ Ct method. Estradiol (E2), progesterone (P4) and IGF-I were evaluated in FFL. In EAF and ATF, FFL E2 concentration was 137 \pm 40 and 21 \pm 6 ng/mL, with E2/P4 ratio of 2 and 0.4, respectively. IGF-I in FFL was 96 \pm 18 and 85 \pm 25 ng/mL for EAF and ATF, respectively. Expression of GHR, IGF-I, IGF-II, SOCS-1 and SOCS-2 mRNA was higher in the CL than EAF and ATF. GHR mRNA expression was 8 times higher, while IGF-I mRNA was 25 times higher in CL than in follicles. SOCS-3 and ER α expression was not different between CL and follicles. No difference between EAF and ATF for the genes studied was found. Regarding follicles, SOCS-2 was correlated to GHR ($r = 0.62$, $P < 0.05$), ER α ($r = 0.87$, $P < 0.0001$) and FFL E2 ($r = 0.55$, $P < 0.05$). In the CL, SOCS-2 was correlated to GHR ($r = 0.85$, $P < 0.05$) and ER α ($r = 0.85$, $P < 0.05$). The IGF-I and SOCS-2 to GHR ratio was lower ($P < 0.01$) in follicles than CL, which indicates that there is more IGF-I and SOCS-2 mRNA production per unit of GHR in the CL. In sum, SOCS-2 and IGF-I mRNA expression in EAF was not different from ATF, although SOCS-2 expression was correlated to FFL E2 concentration. Moreover, GHR effectiveness in stimulating IGF-I seems reduced in the follicle when compared with the CL.

Key words: granulosa cells, GHR, IGF-I

W236 Functional genomics of liver in purebred beef cows in two forage allowances during gestation and lactation period. J. Laporta^{*1}, G. Greif², P. Zorrilla², H. Naya², G. J. M. Rosa³, and M. Carriquiry¹, ¹Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay, ²Instituto Pasteur, Montevideo, Uruguay, ³University of Wisconsin, Madison.

In rangeland conditions, pasture quality and availability vary throughout the year affecting energy balance of pregnant and lactating beef cows. A large microarray experiment was conducted using purebred cows (PU; Angus and Hereford) in high (H) and low (L) forage allowances (10 vs. 6 kg DM/100kgLW/d) to study liver gene expression during gestation and lactation periods. Four cows per treatment (PU-H and PU-L) were used. Total RNA was extracted from liver biopsies (-170, -15, +15 and +60 d relative to parturition). Integrity and quality were evaluated using Agilent 2100 Bioanalyzer (RIN 6.4 \pm 0.4). A single-channel microarray analysis was performed using Agilent 4x44K Bovine Gene Expression array. Data was normalized, filtered, and a 2-way ANOVA, to evaluate the effect of time and forage allowances, was performed using GeneSpring (v11.5) Software. Significance levels were adjusted for multiple testing using a false discovery rate of 0.2. Out of 806 differentially expressed genes (DEG), 795 changed only across time (140 \pm 2.5 fold change), and 12 with forage allowance. There were no significant interactions between time and forage allowance. The DEG were clustered to study expression profiles and a Gene Set Enrichment Analysis was performed. More than 15 significant ($P \leq 0.01$) gene sets across time (only for -170 vs. -15 d) with posi-

tive (IGFI-MTOR pathway, spliciosome, and MAPK pathway) and negative (PPAR signaling pathway, triacylglyceride biosynthesis, and pyruvate metabolism, etc.) enrichment scores (ES) were identified. Only PPAR signaling pathway was enriched in -15 vs. +15 d. Steroid metabolism, cholesterol biosynthesis, glucogen and glycogenolysis, among others, were positively enriched for +15 vs. +60 d. Only four genes sets were significantly ($P \leq 0.01$) and negatively enriched when high vs. low forage were compared: amine hormones, glucose SLC sugar transporters, neurotransmitter release and MAPK signaling pathway. These results contribute to understand liver function in regulating alterations in metabolism in response to the level of nutrition and physiological stage in beef cattle along the year on grazing conditions.

Key words: microarrays, liver, grazing

W237 Conjugated linoleic acids (CLA) and lactation related changes of serum amyloid A3 (SAA3) and IL-6 mRNA abundance in different bovine tissues with a focus on different adipose depots.

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The adipokine SAA3 is linked to obesity and insulin sensitivity of adipose tissue (AT) and respective inflammatory response, at least in mice. SAA3 is not related to the plasma concentrations of SAA. In contrast, SAA3 could be found in medium of cultured adipocytes. SAA3 is known to be upregulated by IL-6 in mice. In dairy cows, CLA interfere with energy homeostasis and fat metabolism by milk fat reduction. We hypothesized that the mRNA abundance (Ab) of SAA3 and IL-6 could be affected by CLA or days postpartum. From 25 Holstein heifers, 5 animals were slaughtered on d 1 postpartum. Remaining heifers were randomly allocated to CLA (Lutrell pure, BASF, Germany, n = 10) or control fat supplementation (Silafat, BASF, n = 10) each at 100 g/d. Five animals per group were slaughtered at d 42 or 105. Liver, skeletal muscle, udder, pancreas and fat samples [subcutaneous (Sc) (chest, wither and tail head) and visceral (Vc) (mesenterial, omental and retroperitoneal)] were collected. Total RNA was analyzed by qPCR. Pearson correlation and statistical analysis using the general linear model or non parametric tests were done in SPSS 17 ($P < 0.05$). Decreasing Ab of SAA3 from d 1 to d 42 and 105 was observed in all tissues except mesenterial AT in which SAA3 was constantly expressed. In ScATs and muscle tissue, CLA reduced SAA3 Ab in comparison to control group regardless of time. In general, 2 to 26 fold differences in the SAA3 Ab in different ATs compared with omental AT (highest SAA3 Ab) were observed. IL-6 Ab was reduced from d 1 to d 42 and 105 in ScATs and vice versa in liver. IL-6 Ab was neither affected by CLA nor correlated to SAA3 Ab. In conclusion, a regulatory effect of IL-6 on SAA3 in mice was not supported for dairy cattle. Reduced SAA3 Ab in ScATs and muscle regarding to CLA, might be a hint of local anti-inflammatory effects of CLA and improved insulin sensitivity in these tissues.

Key words: SAA3, IL-6, adipose tissue

W238 Role of nuclear receptors in the metabolism of boar taint compounds in Leydig cells. M. A. Gray^{*} and E. J. Squires, *University of Guelph, Guelph, Ontario, Canada*.

Boar taint is an unpleasant odor and taste caused by accumulation of androstenone and 3-methylindole (3MI, skatole) in fat of male pigs. The objective of this work was to determine the effects of the nuclear receptors constitutive androstane receptor (CAR), pregnane X receptor (PXR), and farnesoid X receptor (FXR) on the production of total 16-androstene steroids (16A) and androgens (AND) from pregnenolone (PREG) in Leydig cells. Treatment of Leydig cells with CAR or PXR agonists resulted in $25.0 \pm 2.7\%$ and $26.0 \pm 2.8\%$ conversion of PREG to 16A, respectively, which was significantly ($P < 0.05$) higher than the $16.0 \pm 2.2\%$ conversion found with the DMSO control. Treatment with a FXR agonist did not significantly affect conversion of PREG to 16A. Conversely, treatment with agonists for CAR, PXR and FXR significantly ($P < 0.05$) decreased the percentage of PREG converted to AND to $40.8 \pm 2.6\%$, $40.2 \pm 1.9\%$, and $46.2 \pm 3.9\%$, respectively, compared with the DMSO control ($58.8 \pm 4.7\%$). Treatment of Leydig cells with 3MI did not significantly alter 16A or AND production. Activation of CAR, PXR, or FXR all resulted in significant upregulation of several genes involved in the conversion of PREG to 16A or AND, as measured using real-time PCR. Although transcription of CYP17A1, the enzyme that converts PREG to both AND and 16A, was not significantly altered, expression of CYB5A and CYB5R1 increased with activation of each receptor. These genes are accessories to CYP17A1 and increase CYP17A1 andien- β synthase activity instead of 17 α -hydroxylase and C17,20 lyase activities, thus favoring 16A production over AND production. Treatment with 3MI resulted in decreased expression of key target genes for each receptor, indicating that skatole acts as an inverse agonist for these receptors. Taken together, the functional and transcriptional effects of transactivation of CAR, PXR, and FXR suggests that activation of these receptors favors the production of 16A, and thereby will result in an increase in boar taint.

Key words: boar taint, nuclear receptor, steroidogenesis

W239 Effects of heat stress on Na⁺/K⁺ATPase activity in growing pigs. S. C. Pearce^{*}, A. J. Harris, N. K. Gabler, and L. H. Baumgard, *Iowa State University, Ames*.

Na⁺/K⁺ATPase pumps are involved with cellular transport processes, osmotic balance and account for a substantial portion of whole body energy expenditure. However, how heat stress (HS) affects Na⁺/K⁺ATPase activity in various tissues has not been studied in a porcine model. Crossbred gilts (n = 48; 35 ± 4 kg BW) were housed in constant climate controlled rooms in individual pens and exposed to 1) thermal neutral (TN) conditions (20°C; 35–50% humidity) with ad libitum intake (n = 18), 2) HS conditions (35°C; 20–35% humidity) with ad libitum intake (n = 24) or 3) pair-fed (PF in TN conditions [PFTN], n = 6: to eliminate confounding effects of dissimilar feed intake [FI]). Pigs were sacrificed at 1, 3, or 7d of environmental exposure and jejunum, longissimus dorsi (LD), and liver samples were collected and analyzed for Na⁺/K⁺ATPase activity. HS pigs had an increase ($P < 0.01$) in body temperature (39.3 vs. 40.8°C), a doubling in respiration (54 vs. 107 bpm) and an immediate decrease in FI (47%; $P < 0.05$) which continued through d7; by design PFTN controls FI mirrored the HS group. Over the 7d period, TN pigs had increased ADG compared with the HS pigs (1.14 vs 0.24 kg/d) while HS pigs lost 2.7 kg BW. Overall, compared with the TN pigs, HS pigs had increased LD Na⁺/K⁺ATPase activity (52%; $P = 0.06$). There was an environment by day interaction in jejunum Na⁺/K⁺ATPase activity as it was markedly increased (175%; $P < 0.05$) on d1, but returned to TN levels by d3. Liver Na⁺/K⁺ATPase activity was not different between the TN and HS pigs. However, PFTN pigs had decreased pump activity compared

with the HS and TN pigs (30%; $P = 0.06$). Irrespective of environment, TN pigs tended to have tissue differences in Na^+/K^+ ATPase activity ($P = 0.08$) as liver activity was lower (27%) compared with jejunum; LD was not different from either tissue. These data indicate HS induces tissue specific increases in Na^+/K^+ pump activity and suggests that ion pump energy expenditure (and presumably total body energetic cost) increases during a thermal load and is more pronounced during acute HS.

Key words: heat stress, energetics, Na^+/K^+ pump

W240 Serum shock did not synchronize clock gene expression in primary bovine hepatocyte cultures. C. A. Kurman*, M. M. McCarthy, L. M. Nemeč, and T. F. Gressley, *University of Delaware, Newark.*

Circadian rhythms are regulated by clock gene expression. In the liver, clock genes are controlled by hormonal and neural signals to result in 24 h patterns of metabolism and nutrient availability. Studying circadian rhythms in vitro requires artificial synchronization of clock gene expression, accomplished by serum shock in monogastric hepatocyte culture systems. Our objective was to determine whether serum shock of primary bovine hepatocyte cultures would synchronize clock gene expression patterns. Monolayer cultures were established from hepatocytes isolated from a 1 wk old Holstein bull calf. At 0 h, cells were serum shocked for 2 h with 50% fetal bovine serum (FBS) followed by 0% FBS for 46 h (treatment = SS) or treated with media containing 10% FBS for 48 h (NSS). Cells were harvested every 4 h and mRNA was isolated. The mRNA levels of 7 clock genes were quantified relative to control genes (RPS9 and β -actin) using qPCR. The experiment was conducted on 2 occasions (Exp. 1 and Exp. 2). Results were statistically evaluated using a model containing fixed effects of treatment, hour, and their interaction. We expected to observe: 1) characteristic 24 h clock gene expression patterns, and 2) more distinctive patterns for SS compared with NSS. Contrary to this hypothesis, anticipated treatment time interactions were observed only for *Per2* in Exp. 2 (Table 1). Although treatment and hour affected various clock genes in each experiment (Table 1), effects of time and treatment were not consistent with published monogastric studies. The serum shock protocol used in these experiments was unsuccessful at synchronizing bovine hepatocyte clock gene expression.

Table 1.

	Gene	Treatment			P-value		
		NSS	SS	SEM	Treat.	Hour	Treat × Hour
Exp. 1	Clock	2.04	1.38	0.22	0.04	0.15	0.63
	Per1	1.7	1.94	0.14	0.25	0.75	0.7
	Per2	2.96	2.32	0.15	0.005	0.001	0.98
	Cry1	2.54	2.22	0.08	0.01	0.05	0.54
	Cry2	2.41	1.84	0.25	0.12	0.87	0.99
	CK1 ϵ	2.38	1.96	0.09	0.002	0.04	0.95
Exp. 2	Clock	2.26	2.06	0.1	0.17	0.18	0.32
	Per1	2.11	2.01	0.08	0.4	0.05	0.49
	Per2	1.93	1.93	0.07	0.91	0.001	0.05
	Cry1	2.41	2.33	0.11	0.61	0.05	0.75
	Cry2	2.1	2.13	0.13	0.87	0.02	0.93
	CK1 ϵ	1.79	1.75	0.11	0.83	0.01	0.31

Key words: clock gene expression, hepatocytes, serum shock

W241 Effect of short-term supplementation in hepatic gene expression in cycling Hereford cows grazing native pastures. F. Bialade¹, A. L. Astessiano*¹, M. P. Grignola¹, J. Laporta¹, C. Viñoles², and M. Carriquiry¹, ¹School of Agronomy, UDELAR, Montevideo, Uruguay, ²Research Institute for Agriculture, Tacuarembó, Uruguay.

Short-term supplementation before the breeding season has been associated with an increase in early pregnancy rate, however, the metabolic changes involved in this response are not clear. To evaluate the impact of a short-term energy supplementation before the breeding season on changes on the hepatic gene expression associated with the GH-IGF axis, adult non-gestating nonlactating Hereford cows ($n = 9$) were used in a randomized block design. Cows (478 ± 11 kg BW, 5.3 ± 0.1 units BCS, scale 1–8) were allocated to 2 groups: control, non-supplemented (CON, $n = 5$) and supplemented (SUP, $n = 4$). The supplement consisted in 2.5 kg of rice barn/cow (90.3%DM, 10%CP, 9%EE, 14% NDF) offered daily for 23 d. All cows grazed on native pastures (forage availability of 603 kg DM/ha). Liver biopsies were obtained at the beginning (d 0) and end (d 23) of the supplementation treatment. The abundance of mRNA of growth hormone receptor (GHR), insulin-like growth factor-I (IGF-I), IGF binding proteins-2 (IGFBP2),-3 (IGFBP3), and insulin receptor (INSR) were measured by real time RT-PCR normalized by hypoxanthine phosphoribosyl-transferase (HPRT) and β -actin as endogenous controls. Means from a mixed model analysis were considered to differ when $P < 0.05$. Cow BCS did not differ between groups and increased, for all cows, 0.6 ± 0.1 units of BCS during the supplementation period. The expression of all 5 genes: GHR (0.66 vs. 0.57 ± 0.10), IGF-I (0.54 vs. 0.89 ± 0.47), IGFBP2 (4.41 vs. 4.41 ± 1.23), IGFBP3 (1.04 vs. 0.80 ± 0.12), INSR (19.10 vs. 31.06 ± 5.30) mRNAs did not differ ($P > 0.15$) between CON and SUP cows. Expression of GHR and IGFBP3 mRNA ($r = 0.62$, $P = 0.005$), GHR and INSR mRNA ($r = 0.45$, $P = 0.056$) and INSR and IGFBP3 mRNA ($r = 0.57$, $P = 0.011$) were positively correlated. We conclude that short-term energy supplementation before the breeding period has no effect on hepatic gene expression associated with the GH-IGF axis in grazing cyclic beef cows in good BCS.

Key words: mRNA, cattle, nutrition

W242 Effect of charcoal extracted bovine follicular and testicular fluids on testes and endocrine organ weights of pre-pubertal male rabbits. A. H. Ekeocha*, *University of Ibadan, Ibadan, Oyo, Nigeria.*

The effect of charcoal-extracted bovine follicular and testicular fluids on testes and endocrine organ weights of pre-pubertal male rabbits was investigated. A total of 15 young male rabbits of various strains with age range of 11–12 weeks old and weighing 1.0 ± 0.2 kg were randomly divided into 3 groups, each consisting of 5 rabbits. The treated groups and the control group were injected intramuscularly with charcoal-extracted bovine follicular fluid, charcoal extracted bovine testicular fluid and charcoal treated distilled water respectively, at the rate of 0.2mL per rabbit on every other day and on 3 different occasions. After administration of the different treatments, testes weight and endocrine organ weights were conducted or carried out. Endocrine organ weights included: thyroid gland, adrenal gland, pituitary gland and kidney. There were no significant differences ($P > 0.05$) in paired testes weights among the 3 different groups. There were no significant differences ($P > 0.05$) in paired thyroid gland, paired adrenal gland between control and treated groups. Contrarily there was a significant increase ($P < 0.05$) in paired kidney weights between testicular fluid treated groups and control and follicular fluid treated groups. There

was a significant decrease ($P < 0.05$) in pituitary gland weight between control and treated groups. Administration of charcoal extracted bovine follicular and testicular fluids to pre-pubertal male rabbits decreased the weights of pituitary gland, which implies that the administration of inhibin to immature rabbits affects the hypo-thalamo-pituitary function as well as block FSH dependent steps in spermatogenesis.

Key words: charcoal extracted, testes, male rabbits

W243 Caspase 3 is upregulated in murine spermatogonia and Leydig cells treated with aflatoxin B₁. K. J. Austin*, R. R. Cockrum, K. L. Speiser, and K. M. Cammack, *University of Wyoming, Laramie.*

Aflatoxin B₁ is hepatotoxic and carcinogenic in a variety of livestock species including cattle, sheep and swine. In addition to poor performance and health, AFB₁ in food sources can lead to infertility in livestock, rodents and humans. The objective of these experiments was to examine the molecular mechanisms associated with reduced fertility using male mice as the model. Specifically, apoptosis and *Caspase 3*, a primary activator of protein cleavage and DNA fragmentation, were investigated. Male ICR mice 4 wk of age were treated with 50 µg/mL BW AFB₁ (n = 9) or placebo consisting of corn oil/ethanol (n = 9) daily for 45 d via IP injection. Following treatment, males were mated to 4 females each for 8 d to determine number of pups sired. Males were then sacrificed for testes collection. Spermatogonia and Leydig cell lines were cultured in vitro and treated with 0, 5, 10 or 20 µg/mL AFB₁ (n = 3 wells/treatment) for 20 h. Effects of treatment were estimated using the GLM procedure of SAS. Message for *Caspase 3*, as analyzed by semi-quantitative real-time RT-PCR, did not differ ($P = 0.20$) in testes of treated males compared with control males. However, spermatogonia treated with 10 µg/mL AFB₁ showed an upregulation ($P = 0.004$) of *Caspase 3* when compared with control (0 µg/mL AFB₁) treated cells. Tunel staining of spermatogonia also showed an increase ($P = 0.03$) in the number of positively stained cells in the treated cultures (5 and 10 µg/mL AFB₁) compared with control cultures. Leydig cells treated similarly showed greater ($P = 0.02$) message for *Caspase 3* at the 10 and 20 µg/mL levels than controls. This is the first report to our knowledge linking aflatoxicosis to apoptosis in reproductive tissues. Results imply that apoptosis may be in part responsible for damage to the testes/testicular cells, resulting in decreased testosterone levels and reduced fertility. More research is needed to determine other components of the apoptotic pathway affected by AFB₁, and whether the upregulation in *Caspase 3* observed in this study occurred as a direct result of insult with AFB₁.

Key words: apoptosis, *Caspase 3*, aflatoxin

W244 Muscle resident adipogenic progenitors are fiber type specific, Pax3/Myf5-independent and form white adipocytes by default. Y. Q. Liu* and S. H. Kuang, *Purdue University, West Lafayette, IN.*

Ectopic accumulation of adipose tissue in skeletal muscles (intermuscular fat, IMF) has been associated with muscle wasting, insulin resistance and diabetes. However, the developmental origin and regulation of postnatal progenitors that give rise to IMF in comparison to other fat depots are unclear. We found that adipogenic progenitors are more enriched in slow than fast muscles. Cre/LoxP-mediated lineage tracing demonstrated that IMF progenitors are exclusively derived from a Pax3/Myf5-independent lineage and readily differentiate into white adipocytes in culture. In contrast, brown adipose tissue progenitors are derived from a Pax3/Myf5 double positive lineage. Interestingly,

progenitors residing in anatomically distinct white adipose depots are all from a Myf5-independent lineage but are heterogeneous for Pax3 lineage dependence. Diphtheria toxin-mediated lineage ablation confirmed that Myf5 cell lineage is required for brown, but not white, adipocyte differentiation. In addition, ablation of Myf5-dependent myogenic lineage enhances adipocyte differentiation, whereas ablation of aP2- dependent adipocyte lineage impairs muscle regeneration in vivo. In old mice, reduced myogenic capacity is accompanied by accumulation of IMF. Finally, we showed that Dlk1 inhibits the differentiation of both white and brown adipocytes. These results demonstrate surprising heterogeneity of tissue-specific adipogenic progenitors and dynamic interactions between skeletal muscle and adipose tissues.

Key words: intermuscular fat, Myf5, Pax3

W245 Effect of urea on interferon-tau response in the bovine endometrium. A. Ahmadzadeh*, T. Davis, and K. Carnahan, *University of Idaho, Moscow.*

High concentrations of blood and uterine urea associated with high dietary protein have been shown to reduce fertility in dairy cows. The objective of this study was to determine the direct effects of urea on protein expression of the endometrial cells of the bovine uteri in response to interferon-tau (IFN-tau) in vitro. The objective was to determine the direct effect of urea on the production of 2 IFN-tau stimulated proteins, ISG15 and Mx1 in bovine endometrial (BEND) cells. Bovine endometrial cells were cultured to 80% confluency and treated with media containing 0, 5, 7.5, or 10 mM urea for 24 h. Subsequently, BEND cells were challenged with 0 or 10,000 antiviral units of recombinant IFN-tau and cells were incubated for an additional 24 or 36 h in media containing 0, 5, 7.5, or 10 mM urea. Cells were in culture for the same period of time regardless of treatment and then harvested. BEND cells were lysed with MPER and the protein concentrations determined by the Bradford assay. Proteins were separated by SDS-page and subjected to Western blot analysis and immunoblotting to assess the production of Mx1 and ISG15. Based on optical density of the images on x-ray film from a chemiluminescent signal, IFN-tau increased ($P < 0.01$) Mx1 and ISG15 production regardless of treatment after 24 and 36 h. There was no effect of urea treatment or urea by IFN-tau interaction on Mx1 and ISG15 production after 24 ($P = 0.9$) or 36 h ($P = 0.4$) of culture. Moreover, there was no effect of either 24 or 36 h or time by urea treatment interaction on the production of Mx1 and ISG15, in response to IFN-tau. These results show that there is no disruption of IFN-tau-stimulated Mx1 or ISG15 production, when BEND cells were exposed to various concentrations of urea in vitro.

Key words: urea, interferon-tau, bovine endometrial cells

W246 Short-term supplementation and temporary weaning on metabolic and endocrine parameters in anestrus and cyclic Hereford cows grazing native pasture. A. L. Astessiano*¹, L. Veloz^{1,2}, C. García Pintos^{1,2}, M. E. Trobo^{1,2}, F. Bialade¹, C. Viñoles², and M. Carriquiry¹, ¹*School of Agronomy, UDELAR, Montevideo, Uruguay,* ²*National Research Institute for Agriculture, Tacuarembó, Uruguay.*

Two experiments were conducted to study the effect of a short-term supplementation (Exp. 1 and 2) and temporary weaning (Exp. 1), before initiation of the breeding period, on metabolic and endocrine parameters in grazing beef cows. In Exp. 1 primiparous beef cows (n = 32, 3.6 ± 0.02 BCS) in anestrus were used in a randomized block design with a 2 × 2 factorial arrangement of short-term supplementation (non-

supplemented, CON vs. supplemented, SUP) and temporary weaning (with vs. without). In Exp.2, adult non-gestating nonlactating beef cows ($n = 15$, 5.3 ± 0.1 BCS) were used in a randomized block design with 2 treatments: CON vs. SUP. The supplement consisted in 2.5 kg of rice bran/cow (90.3% DM, 10% CP, 9% EE, 14% NDF) offered daily for 23 d (Exp. 1 and 2) and temporary weaning was performed by applying nose plates to calves for 14 d (Exp.1). Blood samples were collected 3 times a week during the treatments. Means from mixed analyses differed when $P < 0.05$. In Exp.1, cow BCS was not affected by treatments. Insulin concentrations were greater ($P < 0.01$) in temporary weaned than suckled cows (2.31 vs. 1.29 ± 0.21 uU/mL), but plasma glucose and cholesterol did not differ among groups. Concentrations of NEFA were greater ($P = 0.04$) in SUP-suckled than in SUP-temporary weaned and CON cows (0.48 vs. 0.33 ± 0.05 mmol/L). In Exp.2, BCS did not differ between groups and increased 0.6 ± 0.1 units during the period evaluated. Insulin (2.89 vs. 3.80 ± 0.55 uU/mL) and glucose (1.1 vs. 1.2 ± 0.05 mmol/L) concentrations were lower ($P = 0.04$) in SUP than CON cows but this effect was most evident in wk 2 and 3. Plasma NEFA did not differ between treatments but cholesterol was greater ($P = 0.01$) in SUP than CON cows (281.9 vs. 234.6 ± 11.3 mg/dL). Metabolic/endocrine changes reflected a better energy balance in short-term supplemented and temporary weaned primiparous cows in anestrus whereas short-term supplementation in cycling cows with good BCS altered glucose/insulin metabolism.

Key words: cattle, nutrition, hormones

W247 Liver gene expression of GH-IGF1 axis and fatty acid metabolism genes of beef cows on grazing conditions. I: Winter-gestational period. J. Laporta*, A. L. Astessiano, V. Gutierrez, A. C. Espasandín, P. Soca, and M. Carriquiry, *Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay.*

In spring-calving cows on rangeland conditions pregnancy occurs in winter, period of limited forage availability, affecting the energy balance of beef cows. Adult pregnant cows ($n = 32$) in a factorial arrangement of genetic group (Angus and Hereford, vs. their crosses; PU vs. CR) and forage allowances (6 vs. 10kgDM/100kgLW/d; LO vs. HI) were used to evaluate the hepatic expression of somatotrophic axis genes (*insulin like growth factor 1*, IGF1; *IGF1 binding protein 3 and 2*, BP3, BP2; *growth hormone receptor*, GHR; *GHR isoform-1A*, GHR1A), fatty acid oxidation genes (*acyl-CoA oxidase-1 palmitoyl*, ACOX1; *acyl-CoA dehydrogenase very long chain*, ACADVL), *peroxisome proliferator activated receptor- α* (PPARA) and, *fibroblast growth factor-21* (FGF 21). Means from a mixed model were considered to differ when $P \leq 0.05$. Liver biopsies were collected in May, August, September and, October (110 ± 10 , 210, 240 and 270 ± 3 d of gestation, dgest). Expression of GHR, PPAR, and FGF21 mRNA were not affected ($P > 0.18$) by any of the factors. Abundance of GHR1A and IGF1 mRNA decreased from 110 to 270 dgest (20 to 9 ± 3 ; 1.6 to 0.9 ± 0.02 , respectively; $P < 0.013$), and BP3 tended ($P = 0.10$) to decrease only at 210 dgest to return thereafter to initial values. The BP2 mRNA increased ($P = 0.002$) at 240 dgest and remained elevated until 270 dgest. Abundance of ACOX mRNA increased throughout gestation in winter, and ACADVL mRNA increased at 240 dgest, returning to initial values at 270 dgest ($P < 0.01$). Expression of BP3, BP2 and ACOX1 mRNA during winter-gestation also depended on cow genetic group ($P < 0.045$). The BP3 mRNA decreased at 210 and 240 dgest in PU, whereas it increased at 240 dgest in CR cows. The BP2 mRNA was greater in PU than CR cows at 110 dgest, and BP2 mRNA increased in both groups (PU at 270 and CR at 240 dgest). The ACOX1 mRNA increased earlier in gestation in PU (210 dgest) than

CR cows (240 dgest). Dynamics of the GH-IGF1 axis genes and the increased expression of β -oxidation genes in the liver reflect changes required to meet energy demands of pregnancy during winter in beef cows on grazing conditions

Key words: somatotrophic axis, energy balance, cattle

W248 Liver gene expression of GH-IGF1 axis and fatty acid metabolism genes in beef cows on grazing conditions. II: Peripartum and lactation period. J. Laporta*, A. L. Astessiano, V. Gutierrez, A. C. Espasandín, P. Soca, and M. Carriquiry, *Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay.*

Beef cows in rangeland conditions are subjected to climate variations that affect pasture growth and variability as cow physiological stage changes from pregnancy to calving and lactation, altering their energy balance. Adult pregnant cows ($n = 32$) in a factorial arrangement of genetic group (Angus and Hereford, vs. their crosses; PU vs. CR) and forage allowances (6 vs. 10kgDM/100kgLW/d; LO vs. HI) were used to evaluate the hepatic expression of somatotrophic axis genes (*insulin like growth factor 1*, IGF1; *IGF1 binding protein 3 and 2*, BP3, BP2; *growth hormone receptor*, GHR; *GHR isoform-1A*, GHR1A), fatty acid oxidation genes (*acyl-CoA oxidase-1 palmitoyl*, ACOX1; *acyl-CoA dehydrogenase very long chain*, ACADVL), *peroxisome proliferator activated receptor- α* (PPARA) and, *fibroblast growth factor-21* (FGF 21). Means from a mixed model analysis were considered to differ when $P < 0.05$. Liver biopsies were collected at -15 ± 3 , 15 ± 3 and 60 ± 3 d relative to parturition, dpp). Expression of IGF1, GHR and, GHR1A were not affected ($P > 0.45$) by any of the factors evaluated. The BP3 and BP2 mRNA abundance decreased ($P < 0.015$) from -15 to 15 dpp, to return then to initial values at 60 dpp. The BP3 mRNA was also affected by the interaction between genetic group and time ($P = 0.04$), while it was not affected in CR cows, it decreased markedly at 15 dpp in PU cows. The expression of BP2 mRNA tended to be greater ($P = 0.09$) in LO than HI cows. The mRNA abundance of 2 key genes involved in fatty acid oxidation varied across time; ACADVL mRNA tended to increase ($P = 0.10$) at 60 dpp, whereas ACOX1 mRNA decreased ($P = 0.004$) from -15 to 15 dpp to return to elevated initial values at 60 dpp. Hepatic PPARA and FGF21 mRNA were not affected ($P > 0.60$) by any of the factors evaluated. We describe the dynamic of the GH-IGF axis during the peripartum and lactation period in beef cows on grazing conditions. Only IGF1 mRNA abundance was altered. The increase of ACADVL mRNA and the elevated levels of ACOX mRNA can reflect the need to oxidize fatty acids during postpartum period to meet energy demands of lactation.

Key words: somatotrophic axis, fatty acid oxidation, cattle

W249 Uterine gene expression in beef cows grazing different forage allowances of native pastures. M. Carriquiry*¹, F. Bialade¹, M. P. Grignola¹, P. Soca¹, A. C. Espasandín¹, C. Viñoles², and A. Meikle³, ¹*School of Agronomy, UdelaR, Montevideo, Uruguay,* ²*National Research Institute for Agriculture, Tracuarembó, Uruguay,* ³*School of Veterinary Sciences, UdelaR, Montevideo, Uruguay.*

The aim of this study was to evaluate the effect of long-term nutrition at 2 different forages allowances of native pastures on uterine gene expression in beef cows. Adult cows (Angus, Hereford and F1 cross-bred) were used, from May 2007 to May 2010, in a complete randomized block design with 2 forage allowances throughout the year (6 vs. 10 kgDM/100kgBW/d; LO vs. HI). At the end of the third year, at 178 ± 15 d postpartum, cows were synchronized with 2 prostaglandin (PG)

injections 11 d apart and slaughtered 32 ± 1 h after the last PG injection. Uterine tissue from the middle third of the uterine horn ipsilateral to the corpus luteum was collected from all cows that had at least 2 previous ovulations ($n = 8$ and 6 for HI and LO, respectively). Relative expression of estrogen (ER α) and progesterone (PR) receptors, growth hormone receptor (GHR), insulin-like growth factor-I (IGF-I), IGF-II, IGF receptor-1 (IGFR1), IGF binding proteins (IGFBP) -2, -3, -4, -5, and -6 mRNA was determined using SYBR-Green real time PCR and normalized to the expression of hypoxanthine-phosphoribosyltransferase and β -actin mRNA. Data were analyzed with a mixed model that included forage allowance and block as fixed and random effects, respectively. At slaughter, cow BCS did not differ ($P = 0.318$) between groups and averaged 3.9 ± 0.08 (scale 1–8). Expression of ER α , PR, GHR, IGF-I, IGF-II, IGFBP3, IGFBP5, and IGFBP6 mRNA did not differ ($P > 0.128$) between forage allowances. However, uterine IGFBP2 tended ($P = 0.092$, 0.30 vs. 0.15 ± 0.08) to be greater and IGFBP4 mRNA was greater ($P = 0.017$, 2.52 vs. 1.25 ± 0.97) in LO than HI cows. Uterine GHR mRNA was correlated ($P < 0.017$) to ER α ($r = 0.65$), IGFBP4 ($r = 0.60$) and IGFBP5 ($r = 0.69$) mRNA while IGF-I mRNA was negatively correlated ($P = 0.043$, $r = -0.53$) to IGFBP5 mRNA and IGF-II mRNA was positively correlated ($P = 0.023$, $r = 0.58$) to IGFBP6 mRNA. Nutritional plane may influence IGF availability in the uterus of beef cows indirectly through changes in expression of IGFBPs.

Key words: cattle, grazing, reproduction

W250 The effect of leptin on primary cultured adipocytes of pigs. J. Liang, X. Zhang, Y. Zheng, S. Pan, R. Zhao, and X. Yang*, Nanjing Agricultural University, Nanjing, P. R. China.

To investigate the effect of leptin on primary cultured adipocytes of pigs and the possibly mechanism mediated by perilipin. SV cells were separated from subcutaneous adipose tissue of weaned piglet. Cells were cultured to 80% confluence followed by differentiation for 3 d, then treated with 10–8 M and 10–7 M leptin respectively for 4h (short-term treatment) or 48h (long-term treatment). Oil-red O and immunofluorescence histochemistry were used to identify adipocytes, lipid droplets and perilipin. Cultured media were collected for quantization of glycerol content. Perilipin, HSL and ATGL mRNA levels were determined by Real-time RT-PCR. Activity of lipases (HSL and ATGL) was determined. Perilipin and phosphorylated perilipin protein levels were quantitated by Western blot analysis. The results showed that after leptin treatment for 4h, the viability of cells was increased significantly in 10–7 M leptin group; cells in 10–7 M and 10–8 M groups released significantly more glycerol than in the control; Perilipin, HSL and ATGL mRNA expression were significantly increased by 10–7 M leptin treatment, whereas 10–8 M leptin treatment increased the mRNA expression of ATGL only; there was no alteration of lipase activity and perilipin content, and the phosphorylation of perilipin was even not observed in both concentration groups. After leptin long-term treatment for 48h, cells in 10–7 M and 10–8 M groups released significantly more glycerol than in the control; Perilipin mRNA was

downregulated by 10–8 M leptin; the perilipin protein content showed downregulate tendency ($P = 0.1$) in 10–7 M leptin group, whereas the phosphorylated perilipin content were detected significantly higher in both treatment groups. The results indicate that the mechanism of the glycerol release in adipocyte induced by leptin short-term and long-term treatment may be different. The leptin short-term treated influence mRNA expression of related genes, and long-term leptin treatment increased lipolytic activity of adipocytes possibly by activating phosphorylation of perilipin.

Key words: adipocyte, leptin, perilipin

W251 Injection of 100 μ g of GnRH 31 d after AI does not reduce pregnancy loss in lactating dairy cows. A. L. A. Scanavez*, L. G. D. Mendonça, P. R. B. Silva, J. G. N. Moraes, and R. C. Chebel, Department of Veterinary Population Medicine, University of Minnesota, St. Paul.

Objectives of the current study were to determine whether treatment with 100 μ g of GnRH 31 \pm 3 d after artificial insemination (AI) reduces pregnancy losses and whether exposure to heat stress affects this outcome. Lactating cows from 2 dairies were enrolled in the study at 31 \pm 3 d after AI. At enrollment cows were grouped by parity and number of AI and assigned to 1 of 2 treatments in a ratio of 1:2. Cows assigned to the GnRH treatment received 100 μ g of GnRH at 31 \pm 3 d after AI and cows assigned to the control treatment did not receive GnRH. All cows were examined by manual palpation per rectum at 38 \pm 3 d after AI (first pregnancy diagnosis) and those diagnosed pregnant were re-examined 66 \pm 3 d after AI (second pregnancy diagnosis). Data regarding daily temperature and humidity were recorded and temperature humidity index (THI) was calculated from 4 weeks before to 9 weeks after AI. At pregnancy diagnosis 38 \pm 3 d after AI there were 606 pregnant GnRH cows and 1,303 pregnant control cows. No cows were exposed to heat stress (THI > 72) between AI and pregnancy diagnosis and average THI between AI and first pregnancy diagnosis was 53.0 ± 0.1 . Average THI from AI to first pregnancy diagnosis was 58.9 ± 0.1 and average THI between first and second pregnancy diagnosis was 63.9 ± 0.1 . Average THI from AI to the second pregnancy exam was 60.1 ± 0.1 , cows were exposed to 0.5 ± 0.1 week with weekly average THI > 72, and 21.1% of cows were exposed to at least one week of heat stress (weekly average THI > 72). Pregnancy loss from 38 \pm 3 to 66 \pm 3 d after AI was not ($P = 0.42$) affected by treatment (GnRH = 5.9, control = 5.1%). Similarly, site ($P = 0.94$), parity ($P = 0.99$), and exposure to heat stress did not affect pregnancy loss from 38 \pm 3 to 66 \pm 3 d after AI. Average projected 305-d milk yield was $11,218.6 \pm 42.2$ kg and projected 305-d milk yield affected ($P < 0.01$) pregnancy loss from 38 \pm 3 to 66 \pm 3 d after AI because cow in the lower 2 quartiles (Q1 = 2.7 and Q2 = 4.2%) had smaller ($P < 0.07$) incidence of pregnancy loss than cows in the higher 2 quartiles (Q3 = 7.7 and Q4 = 6.9%). Treatment with GnRH 31 d after AI does not reduce pregnancy loss regardless of exposure to heat stress.

Key words: dairy cow, pregnancy loss, GnRH