evaluated. The averages were compared by ANOVA followed by the Tukey's test and data are presented as mean ± SEM. It was found a reduction ($P < 0.05$) on MMP after IVM with L-car 10mM (0.4 ± 0.0^{c}) in comparison to 1 m*M* (1.1 ± 0.1^{ab}) and 5 m*M* (1.0 ± 0.0^{ab}), as well as to 0h (1.0 ± 0.1^{a}); however, no treatment differed from the Control (0.8 ± 0.0^{abc}). In respect to ROS levels, we found an increase ($P < 0.05$) in oocytes matured with 10 m*M* (2.3 ± 0.1^{c}), in comparison to Control (1.8 ± 0.1^{b}), 1 m*M* (1.9 ± 0.1^{b}), 5 m*M* (2.0 ± 0.1^{b}) and 0 h (1.0 ± 0.0^{a}). Bl rates were similar ($P > 0.05$) in all treatments (25.2% ± 3.7 to 37.1% ± 2.7). In conclusion, the reduction on MMP after IVM of bovine oocytes with L-car 10 m*M* was followed by an increase of ROS level. However, there was no influence on their acquisition of capacity to Bl development. Financial support: FAPESP (#2012/10084–4 and #2013/07382–6)

Key Words: L-carnitine, mitochondrial membrane potential, ROS level

T319 Cell proliferation in ovarian follicles in nonpregnant ewes: Effects of plane of nutrition and arginine. Anna T. Grazul-Bilska*, Samantha L. Kaminski, Casie S. Bass, Kaitlyn K. Ebel, and Dale A. Redmer, *North Dakota State University, Fargo, ND.*

The aim of this study was to investigate the role of the NO system in ovarian function by determining if Arg supplementation affects cell proliferation in ovarian follicles in nutritionally compromised ewes. Ewes were stratified by weight and randomly assigned into either control (C; n = 35), overfed (O; 2xC; n = 37), or underfed (U; 0.6xC; n = 36) groups 8 weeks before Arg-treatment. Ewes from each nutritional group were randomly assigned to one of 2 i.v. treatments: saline (~10 mL) or Arg (L-Arg-HCl, 155 µmol Arg/kg of BW) which was initiated on d 0 of the estrous cycle and administered 3 times per day (0700, 1400, 2100 h) until ovary collection at the late-luteal stage of the first estrous cycle, or early or mid-luteal stages of the second estrous cycle. Ovaries were fixed in formalin solution followed by immunohistochemical localization of Ki67 (a marker of proliferating cells), and image analysis of granulosa (G) and thecal (T) layers. Data were analyzed by ANOVA using SAS. During nutritional treatment, C maintained BW, O gained 6 ± 1.2 kg, and U lost 14 ± 1.3 kg. Ki67 was detected in G and T layers, and other ovarian compartments. Cell proliferation in G and T of healthy follicles was affected by stage of the estrous cycle, but not plane of nutrition or Arg-treatment. Cell proliferation was 1.3–1.5-fold greater ($P < 0.001$) at early and mid than late luteal phase. For G and T of healthy follicles, interactions ($P < 0.05$) between plane of nutrition, Arg-treatment and/or stage were detected, demonstrating stimulatory effects of Arg-treatment on cell proliferation at the early luteal phase in O and U, and inhibitory Arg-effects at the mid-luteal phase in O ewes. Cell proliferation was greater ($P < 0.001$) in healthy antral than atretic follicles in all groups (14.4 ± 0.4 vs. 1.9 ± 0.1%). These data show that cell proliferation in G and T layers is affected by plane of nutrition, Arg-treatment and/or stage of the estrous cycle that likely affects follicular function. The mechanism of regulation of ovarian cell proliferation by diet and Arg remains to be elucidated. Supported by USDA-AFRI grant 2011–67016–30174, and Hatch Projects ND01748 and ND01754 to ATGB and DAR.

Key Words: plane of nutrition, arginine, ovary

T320 Comparison of mRNA expression of dominant follicle and follicular cyst in lactating dairy cows. Diego A. Velasco Acosta*[1,2], Tonja Egan[2], Cassandra Skenandore[2], Saige Sulzberger[2], Augusto Schneider[2], Fabio Lima[2], Marcio Corrêa[1], and Felipe Cardoso[2], [1]*Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil,* [2]*University of Illinois, Urbana, IL.*

Follicular cysts are an important disorder affecting 5 to 30% of dairy cows that leads to abnormal estrous cycle behavior and economic losses due to infertility. The aim of this study was to compare the mRNA expression in follicular cells of cows classified either as having a follicular cyst or cycling with dominant follicle (DF). Lactating dairy cows (n = 22) were examined weekly by ultrasound and 11 cows (Holstein, n = 7; Jersey n = 3; and Shorthorn n = 1) were classified as having a follicular cystic (CYS, follicle >25 mm with absence of corpus luteum) whereas 11 cows (Holstein, n = 11) were classified as cycling with a dominant follicle (CON, presence of corpus luteum and dominant follicle, n = 11). Cows were at 94.4 (30 – 382) DIM, milk yield 37.9 ± 12 kg /d, and parity 2.6 ± 1.3. Cows in CON had follicle diameter of 14.10 ± 3 mm whereas cows in CYS had follicle diameter of 35.73 ± 10.2 mm. Follicular fluid from each cow was aspirated and follicular cells were retrieved immediately by centrifugation, frozen in liquid nitrogen and stored at −80°C until RNA extraction. The mRNA expression for *LHCGR, STAR, 3β-HSD, P450scc, CYP19A1, IRS1, IGF, TLR4, TNF, IL1-β, IL8* and *IL6* was measured by real-time PCR. Statistical analysis was performed using the MIXED procedure of SAS. *LHCGR, 3β-HSD, CYP19A1, IRS1* mRNA expression was higher ($P < 0.05$) in CON (1.37 ± 0.4, 5.58 ± 1.2, 1.33 ± 0.2, 1.41 ± 0.3, respectively) than CYS (0.69 ± 0.3, 3.24 ± 0.9, 0.24 ± 0.01, 0.74 ± 0.2, respectively) with breed effect ($P < 0.07$). Expression of mRNA for *IGF, TLR4, TNF* and *IL8* was lower ($P < 0.05$) in CON (0.55 ± 0.07, 0.61 ± 0.2, 0.54 ± 0.3, 0.12 ± 0.01, respectively) than in CYS (4.62 ± 0.7, 3.40 ± 0.5, 1.58 ± 0.3, 0.52 ± 0.04, respectively), with no breed effect ($P > 0.20$). There was no difference ($P > 0.05$) for *STAR, P450scc, IL6* and *IL1-β* mRNA expression between CYS and CON. In conclusion, cows in CYS had lower expression of genes related to steroidogenesis and energy metabolism and greater expression of genes related to inflammation than CON. It seems that an inflammatory response may be involved in the follicular cyst etiology.

Key Words: follicle, cystic, inflammation.

T321 Colony-stimulating factor 2 affects development of the bovine preimplantation embryo differently for females than males. Luiz G. B. Siqueira*[1,2] and Peter J. Hansen[1], [1]*University of Florida, Department of Animal Sciences, Gainesville, FL,* [2]*Embrapa Gado de Leite, Juiz de Fora, MG, Brazil.*

Colony-stimulating factor 2 (CSF2) regulates early embryonic development by modifying the epigenome, reducing apoptosis, and altering ratio of cells in the trophectoderm (TE) and inner cell mass (ICM) of the blastocyst. Previously, CSF2 reduced trophoblast elongation in female embryos but increased elongation in males. Here it was tested whether sexual dimorphism in response to CSF2 can be observed as early as the blastocyst stage. Embryos were produced in vitro using X- or Y-sorted sexed semen (n = 1612 putative zygotes). On d 5 of culture, droplets were supplemented with 5 µL vehicle (control) or 10 ng/mL bovine CSF2. Blastocysts (n = 210) were collected at Day 7 and labeled with a nuclear dye (Hoescht 33342; total cells) and a TE cell marker (CDX2). Number of ICM cells was calculated by subtraction. Statistical analysis was performed using the Proc Mixed procedure of SAS; data represent least squares means ± SEM. Treatment of female embryos with CSF2 increased the proportion of zygotes ($P = 0.0213$) and cleaved embryos ($P = 0.0252$) to become a blastocyst but there were no effects in males ($P > 0.10$). The percent of zygotes becoming blastocysts on Day 7 was 14.7 ± 2.1 vs 21.5 ± 2.1% for control and CSF2 in females and 16.2 ± 2.0 vs 16.3 ± 2.0% in males. There was no effect of CSF2 treatment, sex, or the interaction on the total cell number or number of TE ($P >$ 0.10). There was a tendency ($P = 0.0934$) for ICM number to be less in females (56.2 ± 3.1 vs 61.0 ± 2.9) and the TE:ICM ratio was greater (*P*

= 0.0217) for females (1.64 ± 0.91) compared with males (1.45 ± 0.09). Numerically (but not significantly), CSF2 tended to decrease ICM in females (53.9 ± 3.6 vs 58.6 ± 3.7) but not in males (60.4 ± 3.5 vs 61.5 ± 3.4). There was a tendency for a CSF2 by sex interaction ($P = 0.0955$) for TE:ICM ratio. In females CSF2 increased ratio (1.73 ± 0.11 vs 1.55 ± 0.11), but no effect was observed in males (1.41 ± 0.10 vs 1.50 ± 0.10). In conclusion, CSF2 exerts different responses on development of female and male preimplantation embryos. Consequences of actions of CSF2 on ICM and TE cell differentiation require further investigation. Support: NIH HD080855.

Key Words: embryo, colony-stimulating factor 2, sex

T322 Effect of fertility stressors on transcriptome of peripheral blood leukocytes (PBL) in dairy cows at the onset of conceptus implantation. Eduardo S. Ribeiro*[1,2], Rafael S. Bisinotto[1,2], Fabio S. Lima[1,2], Natalia P. Martinez[1,2], Leandro F. Greco[1,2], William W. Thatcher[1,2], and José E. Santos[1,2], *[1]Department of Animal Sciences, University of Florida, Gainesville, FL, [2]D.H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville, FL.*

Objectives were to investigate changes in transcriptome of PBL occurring at the onset of implantation and how they are affected by fertility stressors. Lactating cows (n = 481) had estrous cycle and ovulation synchronized to receive their first insemination (AI) postpartum. On d 19 after AI, PBL were isolated and mRNA extracted. A subsample of PBL mRNA from 36 cows was subjected to transcriptome analysis using the Affymetrix platform. Pregnancy was diagnosed on d 34 after AI. Two fertility stressors were evaluated, progesterone concentration during development of the ovulatory follicle and clinical uterine diseases (UTD). At the onset of synchronization, experimental design was established to have cows ovulating follicles that grow under low (LP) or high (HP) concentrations of progesterone. In addition, cows were evaluated daily on the first 10 d postpartum for diagnosis of UTD. Statistics was performed using Limma on R and FDR adjustment was used. LP during development of the ovulatory follicle reduced pregnancy per AI (P/AI; 34 vs 53%) and altered the transcriptome of PBL. In the HP group, pregnancy resulted in upregulation of classical interferon stimulated genes (e.g., *IFI6*, *ISG15*, *OAS1Y*); whereas in the LP group, pregnancy resulted in downregulation of a large number of inflammatory response genes (e.g., *HP*, *JUN*, *MYD88*). Particularly distinct transcriptome was observed in LP cows that failed to become pregnant, which indicated an inflammatory state. Cows that suffered from UTD also presented reduced P/AI (33 vs 50%) but only subtle differences in transcriptome, although potentially important. In pregnant cows previously diagnosed with UTD, expression of *OAS1X* was downregulated whereas *CD244* was upregulated compared with pregnant cows not diagnosed with UTD. Fertility stressors were associated with altered PBL transcript profiles at the onset of implantation. Differences observed might represent either a primary cause of subfertility or a consequence of impaired developmental potential of their conceptus and its ability to secrete signaling molecules to modulate the maternal immune system.

Key Words: cow, fertility, leukocyte

T323 Circulating concentrations of bovine pregnancy associated glycoproteins and late embryonic mortality in lactating dairy herds. Ky G. Pohler*[1], Marcos H. Pereira[2], Francisco R. Lopes[2], Jose L. M. Vasconcelos[2], Michael F. Smith[1], and Jon A. Green[1], *[1]Division of Animal Sciences, University of Missouri, Columbia, MO, [2]FMVZ–UNESP, Botucatu, SP, Brazil.*

In cattle, the incidence of late embryonic mortality (EM) has been reported to range from 3.2 to 42.7%. In some cases, the economic consequences of late EM are reported to be greater than that of early EM, because late EM can cause a significant delay in conception date. Although considerable effort has been directed toward understanding the causes of early EM, relatively little is known about the causes or mechanisms associated with late EM. The objectives of this experiment were as follows: (1) to determine the association between circulating concentrations of pregnancy associated glycoproteins (PAGs) and late EM in lactating dairy cattle following fixed-timed artificial insemination (TAI) on d 0 or fixed-timed embryo transfer (TET) on d 7, and (2) to identify a circulating concentration of PAGS on d 30 below which late EM would be likely to occur. Cows were diagnosed pregnant on d 30 of gestation based on presence of a fetal heartbeat and reconfirmed on d 60 of gestation. Late EM occurred when a cow had a viable embryo on d 30 of gestation but not on d 60 following TAI or TET. Only pregnant cows were included in the analysis (TAI-maintained, n = 413; TAI-EM n = 77; TET-maintained, n = 238; TET-EM, n = 47) which was subjected to Proc Glimix procedures of SAS. Cows that were pregnant at d 30 of gestation and maintained the pregnancy until d 60 had significantly greater ($P < 0.05$) circulating concentrations of PAGs at d 30 of gestation compared with cows that experienced late EM between d 30 and 60 of gestation in both TAI and TET. A receiver-operating characteristic curve was generated using MedCal Software to determine circulating concentration of PAGs on d 30 that should predict EM with ≥95 accuracy in both TAI and TET. Based on positive and negative predicative value analysis, a circulating concentration of PAGs below 1.4 ng/mL (TAI) and 1.85 ng/mL (TET) was 95% accurate in predicting EM (between d 30 - d 60) at d 30 of gestation. In summary, PAGs may provide a good marker for predicting EM between d 30 to 60 of gestation and may be able to accurately predict cows that will undergo late EM for the purpose of investigating mechanisms leading to late EM.

T324 Etiology of early pregnancy losses in Holstein dairy cows based on serum pregnancy-associated glycoprotein and progesterone concentrations. Maria J. Fuenzalida*, Paulo D. Carvalho, Milo C. Wiltbank, Pamela L. Ruegg, and Paul M. Fricke, *University of Wisconsin-Madison, Madison, WI.*

Our objective was to describe the mechanism and timing of pregnancy losses (PL) in cows after the first timed artificial insemination (TAI). A total of 136 cows that experienced PL were included in a matched case-control study. Cases were obtained from 3,164 cows from 4 dairy farms enrolled in a prospective cohort study. Cows with pregnancy-specific protein B (PSPB) ≥ 0.3 ng/mL on d 25 after TAI and were open based on transrectal ultrasonography 27 to 32 d (PG1) were defined as early PL (n = 49 cows). Cows that were pregnant at PG1 but open at subsequent evaluations were considered later PL (n = 87 cows). Cows that remained pregnant during the study period (from TAI to 90 d after TAI) were defined as Controls (n = 266 cows). Two Control cows were matched to each PL cow based on days in milk and parity. Progesterone (P4) and PSPB were measured weekly from 10 d before TAI until a cow was diagnosed open or remained pregnant and reached 90 d after TAI. Week of PL and cause of PL (embryonic death [ED] or corpus luteum regression [CLR]) was determined from weekly PSPB using a cutoff of the lowest PSPB concentrations of Controls (from 25 to 88 d after TAI) and weekly P4 concentrations. Data were analyzed by *t*-test, chi-squared and linear regression. For early PL, 30.6% (15/49), 16.3% (8/23) and 53.1% (26/49) were due to ED, CLR and undefined causes, respectively. For cows with later PL, 37.9% (33/87), 48.3% (42/87), and 13.8% (12/87) were due to ED, CLR and undefined causes ($P = 0.04$). Mean average days for occurrence of PL based on PSPB concentration

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