034 Gametes Biology, Embryology and Fetal Development

Components of *Biserrula pelecinus* that affect *in vitro* maturation of ovine oocytes, and subsequent fertilisation and embryo development

A.A. Amir*[1,3], A.A. Algreiby[5], J.M. Kelly[4], D.O. Kleemann[4], Z. Durmic[1,2], G.R. Flematti[5], D. Blache[1,2], G.B. Martin[1,2]

[1]School of Agriculture and Environment, University of Western Australia, Crawley, Western Australia, Australia; [2]UWA Institute of Agriculture, University of Western Australia, Crawley, Western Australia, Australia; [3]Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Selangor, Malaysia; [4]South Australian Research and Development Institute, Turretfield Research Centre, Rosedale, South Australia, Australia; [5]School of Molecular Science, The University of Western Australia, Crawley, Western Australia, Australia.

New pasture legumes need to be tested for deleterious effects on reproduction in grazing livestock. Measurement of these effects in grazing animals is difficult, so *in vitro* techniques are preferred for initial screening. Using ovine oocytes and embryos, we have previously observed the effects of the isoflavones responsible for clover disease and the effects of a crude extract of *B. pelecinus*. Here, we go to the next stage by attempting to identify the plant secondary metabolites produced by *B. pelecinus* that could be responsible for the effect. Experiment 1: *B. pelecinus* was extracted with 1:1 (v/v) MeOH/$CHCl_3$ and the extract was fractionated using rapid silica filtration (RSF) with solvents of varying polarities; dried fractions were added, at final concentrations of 0, 100 or 200 µg mL^{-1}, to the medium used for *in vitro* maturation of cumulus-oocyte complexes (COCs) derived from abattoir-sourced adult ewe ovaries; matured oocytes were then taken through *in vitro* fertilisation and embryo culture so we could quantify embryo development (cleavage rate, blastocyst rate, hatching rate, blastocyst efficiency) and total blastocyst cell number (TCN). Data for embryonic development were analysed using CATMOD in SAS while TCN data were analysed using the GLM procedure in SAS. Of seven fractions tested, one fraction reducing cleavage rate by 9.8% and three fractions reduced blastocyst development by 14-22%. However, one of the active fractions (designated BP6) at 200 µg mL^{-1} increased hatching rate by 20% ($P<0.05$). Experiment 2: fraction BP6 was further fractionated by semi-preparative HPLC and loliolide was clearly the most abundant compound. Pure loliolide at 25 µg mL^{-1} increased hatching rate ($P<0.05$), consistent with the effects observed with fraction BP6. All oocytes supplemented with *B. pelecinus* fractions and loliolide reached the final stage of embryo development, blastocyst hatching, with no effect on TCN. We conclude that the ability of *B. pelecinus*, present during *in vitro* oocyte maturation, to improve fertilisation and embryo development, is at least partly due to the presence of loliolide. We now need to test the concept *in vivo*.

035 Gametes Biology, Embryology and Fetal Development

Effect of supplementation with Cysteine, Glycine and Glutamate during *in vitro* fertilization of bovine oocytes

J.P. Anchordoquy*, J.M. Anchordoquy, N. Nikoloff, A.C. Carranza, A.M. Pascua, N.A. Farnetano, C.C. Furnus

IGEVET, Instituto de Genética Veterinaria "Ing. Fernando N Dulout" (UNLP-CONICET-CONICET LA PLATA, La Plata, Buenos Aires, Argentina.

The constitutive amino acids involved in glutathione synthesis are glycine (Gly), glutamate (Glu) and cysteine (Cys). In a previous study, we demonstrated that the supplementation with Cys, Gly and Glu during *in vitro* maturation of bovine oocytes improves its subsequent developmental capacity (Furnus *et al*., 2008. Metabolic requirements associated with GSH synthesis during in vitro maturation of cattle oocytes. Anim Reprod Sci. 109(1-4):88-99). The aim of this study was to evaluate the effect of Cys, Gly and Glu during *in vitro* fertilization (FIV). Cumulus–oocyte complexes (COC) obtained from cattle ovaries collected in an abattoir were matured *in vitro* in TCM 199 medium containing 10% FCS and hormones. The incubations were performed at 39 ºC in an atmosphere of 5% CO_2 in air with saturated humidity for 24 h. Then matured COC were washed twice in HEPES-TALP, and placed into 50 µl drops of IVF under mineral oil. COC were *in vitro* fertilized in a) TALP medium alone (Control) or supplemented with b) 0.6 mM Cys + 0.9 mM Glu + 0.6 mM Gly (TALP aa); c) 0.6 mM Gly + 0.9 mM Glu (TALP Gly+Glu); d) 0.6 mM Cys + 0.9 mM Glu (TALP Cys+Glu); and e) 0.6 mM Cys + 0.6 mM Gly (TALP Cys+Gly). To determine the effect of amino acids on subsequent embryo development, the presumptive zygotes were cultured for 9 days in SOFm medium. Cleavage rates were recorded 48 h after insemination. At the end of incubations, stages of embryo development were evaluated with an inverted microscope. For this purpose, 440 COC were matured in five replicates. A completely randomized block designs were used. Statistical model included the random effects of block and the fixed effect of treatment. Cleavage, blastocyst and hatching rates were analyzed by logistic regression using GENMOD procedure (SAS Institute). Percentages of cleavage (CL) and blastocyst (BL) for Control (CL: 75.88 - BL: 36.62%); TALP Gly+Glu (CL: 65.21- BL: 32.64%); TALP Cys+Glu (CL: 59.18- BL: 21.08%), TALP Cys+Gly (CL: 71.37 - BL: 35.22%) and TALPaa (CL: 57.05 - BL: 19.66%) were significantly lower in both, TALP Cys+Glu and TALPaa, compared with Control ($P<0.05$). On the other hand, no differences were found in hatching rates among the different treatments (62.21, 60.3, 40.84, 56.17 and 39.87% for Control, TALP Gly+Glu, TALP Cys+Glu, TALP Cys+Gly and TALPaa, respectively; $P>0.05$). In conclusion, IVF medium supplementation with Cys, Gly and/or Glu did not show an improvement effect on subsequent embryo development. On the contrary, the combination of Cys + Glu decreased cleavage and blastocyst rates.

036 Gametes Biology, Embryology and Fetal Development

Heat shock compromises bovine oocyte endoplasmic reticulum reorganization during *in vitro* maturation

L.B. Latorraca*[1], W.B. Feitosa[2], F.F.P. Lopes[2]

[1]Institute of Biosciences - UNESP, Botucatu, São Paulo, Brazil; [2]Federal University of São Paulo - UNIFESP, Diadema, São Paulo, Brazil.

Oocyte maturation is characterized by a dynamic reorganization of the endoplasmic reticulum (ER) increasing the ability of the oocyte to release Ca^{2+} during fertilization (Zhang *et al*., 2013. Maternal diabetes causes abnormal dynamic changes of endoplasmic reticulum during mouse oocyte maturation and early embryo development. Reproductive Biology and Endocrinology. 11:31). There is evidence that oocyte maturation is susceptible to disruption by elevated temperature compromising the capacity of the oocyte to undergo adequate fertilization and embryonic development. Heat shock (HS) during in vitro maturation (IVM) has been shown to damage the oocyte cytoskeleton (Rodrigues *et al*., 2016. Thermoprotective effect of insulin-like growth factor 1 on in vitro matured bovine oocyte exposed to heat shock. Theriogenology. 86:2028-2039) which may affect ER migration. Therefore, the objective of this study was to determine whether exposure of bovine oocytes to a moderate HS during IVM compromises ER reorganization. Grade I and II cumulus-oocyte complexes (COCs) obtained from slaughterhouse ovaries were examined immediately after collection (immature oocyte - 0 h) or subjected to IVM at control (38.5°C) and heat shock (40°C) for 24 h. COCs from all experimental groups were denuded by vortex in 0.1% hyaluronidase and washed twice in TCM 199 HEPES. Denuded oocytes were incubated in TCM 199 HEPES containing 100 nM ER-Tracker Red for 30 min on humidified chamber at 38.5°C and 5% CO_2. After incubation, oocytes were washed in TCM 199 HEPES and transferred to slides mounted with coverslips. Samples were analyzed in Zeiss Imager.A2 epifluorescence microscope. The most prevalent ER distribution categories were: uniform ER distribution through the oocyte cytoplasm for immature control oocytes and cortical ER localization for mature control oocytes. Data were analyzed by least-squares analysis of variance using the General Linear Models procedure of SAS. In vitro maturation at 38.5°C increased ($P < 0.005$) the percentage of oocytes with cortical ER distribution as compared to 0h (65.0 + 6.5% vs 10.0 + 6.5%, respectively). Exposure of oocytes to 40°C during IVM decreased ($P < 0.05$) the percentage of oocytes with cortical ER distribution as compared to 38.5°C (29.1 ± 8.0% vs 65.0 ± 6.5% respectively). Moreover, the percentage of oocytes with uniform ER distribution through the cytoplasm was 74.2 ± 1.0, 19.8 ± 1.0 and 43.4 ± 1.3% for immature oocytes and oocytes matured at 38.5ºC and 40ºC, respectively. In conclusion, moderate HS of 40°C during 24 h IVM reduced ER reorganization to the cortical region of the oocyte which could negatively impact fertilization. Previous experiments demonstrated that HS during IVM reduced cortical microfilament organization in bovine oocytes. Therefore, it is possible that heat-induced cytoskeletal changes mediate the negative effect of HS on ER distribution. This research was supported by Sao Paulo Research Foundation (FAPESP; 2017/13082-6) and Coordination for the Improvement of Higher Level Education – Personnel (CAPES) - Program attraction of young Talents (CSF-PAJT - 88887.068701/2014-00).

037 Gametes Biology, Embryology and Fetal Development

Influence of sodium pump on ram sperm parameters

D.R. Câmara*[1], A.S. Oliveira[2], A.M. Batista[2], R.A.J. Silva[2], L.C.P. Arruda[2], M.M. Monteiro[2], J.A.S. Costa[2], M.M.P. Guerra[2]

[1]Animal Reproduction Laboratory, Federal University of Alagoas, Viçosa-AL, Brazil; [2]Andrology Laboratory, Federal Rural University of Pernambuco, Recife-PE, Brazil.

The sodium pump (NaP) is a transmembrane protein affecting sperm motility parameters (mouse and human) and capacitation (bulls). However, there are few reports regarding the role of NaP in ram sperm. Preliminary data indicate that NaP is localized mainly in the middle piece of ram sperm flagellum (unpublished data). The goal of this study was to determine the effect of NaP on ram sperm parameters. Four semen collections (48-h intervals) were performed in five rams using artificial vagina. Fresh ejaculates from the same day were pooled, split in two tubes and centrifuged (600 × g, 37°C for 10 min) with TALPm (1:10, v:v) to remove seminal plasma. Thereafter, samples were re-suspended (50 × 10^6 sperm/mL) in TALPm + 2.5% estrous sheep serum with (Treated) or without (Control) addition of NaP inhibitor ouabain (10^{-4} M) and incubated for 3 h (38.5°C). Flow cytometric analyses were performed (Amnis ImageStreamx Mark II, Millipore Corp.) to evaluate acrosomal and plasma membrane integrity (FITC-PNA and IP), plasma membrane stability in viable cells (M540 and Yo-Pro-1), and mitochondrial membrane potential (JC-1). Kinematic parameters (total (TM) and progressive motility (PM), linearity (LIN), straightness (STR), curvilinear velocity (VCL), progressive velocity (VSL), path velocity (VAP), amplitude of lateral head displacement (ALH), and beat frequency of the tail (BCF) were assessed with a CASA system (SCATM; Microptics, S.L., Version 5.1). Differences between Control and Treated samples, at 0 and 3 h of incubation, were assessed using ANOVA, followed by Mann-Whitney pairwise with PAST software Version 3.18. For all analyses, untransformed data were used and $P < 0.05$ was considered significant. At 0 h of incubation, all endpoints assessed were similar between Treated and Control samples ($P > 0.05$). There was no influence of treatment or incubation time on percentage of live sperm with membrane stability ($P > 0.05$). The percentage of sperm with intact acrosomal and plasma membranes was higher in Treated than Control group ($P < 0.05$) after 3 h of incubation. Ouabain reduced the percentage of sperm with high mitochondrial membrane potential (HMMP) after 3 h compared to 0 h ($P < 0.05$), whereas in Control samples, HMMP was similar throughout incubation. There was no influence of treatment or incubation on LIN, STR, VAP and BCF. However, whereas in Control samples there was a significant reduction of MT, but not MP, treatment with ouabain led to the opposite response, with significant reduction in MP but not MT, and no difference between treatments at the same incubation time. In both Treated and Control samples, incubation reduced VCL ($P < 0.05$), but there was no difference between Control and Treated samples within time. Treated samples had reduced VSL after incubation ($P < 0.05$), with no change in Control samples. Conversely, ALH was similar in Treated samples at 0 and 3 h, but there was a significant reduction in Control. In conclusion, NaP affected kinematic parameters of ram sperm and, as an ionic channel. Effects were attributed to its influence on mitochondrial function. FACEPE-financial support.

038 Gametes Biology, Embryology and Fetal Development

Ghrelin antagonist: possible solution for *in vitro* oocyte maturation from cows in negative energy balance

A. Carranza Martin*[1], N. Nikoloff[1], J.M. Anchordoquy[1], A. Pascua[1], J.P. Anchordoquy[1], A.E. Relling[2], C. Furnus[1]

[1]Veterinary Genetic Institute "Ing. Fernando Noel Dulout", La Plata, Buenos Aires, Argentina; [2]Animals Sciences Ohio State University, Wooster, Ohio, EEUU.

Ghrelin is a gut hormone released when cows are in negative energy balance (NEB) with adverse effects on oocyte in vitro maturation (IVM) (Sirini *et al*., 2017. Zygote, 25:601-611). The aim of this study was to investigate the effect of ghrelin antagonist (Lys3-GHRP-6). For this purpose, cumulus oocyte-complexes (COC) were obtained from slaughterhouse ovaries; and then maturated for 24h in TCM199 with 0.4% BSA, FSH (1µg/mL) and estradiol 17-beta (1µg/mL). The COC were matured in IVM medium: a) alone (Control: C); or supplemented with b) 60pm/mL ghrelin (G); c) 60pm/mL ghrelin+20pM/mL Lys3-GHRP-6 (ghrelin antagonist 1: GA1); d) 60pm/mL ghrelin+60pM/mL Lys3-GHRP-6 (ghrelin antagonist 2: GA2); e) 60pm/mL of ghrelin+100pM/mL Lys3-GHRP-6 (ghrelin antagonist 3: GA3). After IVM oocyte nuclear maturation was analyzed by Hoechst 33342, cumulus expansion area (CEA) was calculated with microscopic photographs by ImageJ, cumulus cells (CC) viability was evaluated by FDA, CC apoptosis and necrosis was classified by Anexina V and propidium iodide (Pläsier *et al*., 1999. J Immunol Methods, 229:81-95) and CC DNA damage was estimated by comet assay. Results of oocyte nuclear maturation, CC viability and rates of apoptosis and necrosis were analyzed by logistic regression using GENMOD procedure, and CEA and genetic damage index were analyzed by mixed model (SAS Institute, Cary, NC, USA). There were not differences among groups in CEA and nuclear maturation rates at any IVM condition analyzed ($P>0.1$). On the other hand, CC viability was significantly higher in C than G or ghrelin plus high ghrelin antagonist supplementation (C 137.39±9.07 vs G 95.5±9.07, GA2 101.52±9.3 and GA3 96.68±9.04; $P<0.01$). However, no differences were observed between C and GA1 ($P>0.1$; 124.34±9.07 vs. 137.39±9.07, respectively). Apoptosis was not significant different between treatments. However, there was a significant difference in CC necrosis between C and G ($P<0.01$; 15.5% vs 38.8%), while C results were similar to GA1, GA2 and GA3 ($P>0.1$; 15.5%, 13.5%, 17%, and 14.3%, respectively). Comet assay data demonstrated that genetic damage index (GDI) was significantly higher in G ($P<0.01$), while C, GA1 and GA2 showed similar GDI. In addition, GA3 presented the lowest GDI ($P<0.01$). In conclusion, the presence of ghrelin antagonist during bovine IVM prevented negative ghrelin effects on oocyte maturation, and GA3 might be a protector to decrease DNA damage.

039 Gametes Biology, Embryology and Fetal Development

Oxidative stress in newborn calves with intrauterine growth retardation is associated with a deficiency of selenium and copper

A.E. Chernitskiy*[1], V.A. Safonov[2]

[1]All-Russian Research Veterinary Institute of Pathology, Pharmacology and Therapy, Voronezh, Russia;
[2]Vernadsky Institute of Geochemistry and Analytical Chemistry, Moscow, Russia.

Intrauterine growth retardation (IUGR) is defined as the discrepancy between the size of the embryos and fetuses and the term of their gestation (Wu *et al*., 2006). The immature antioxidant defense system of newborn animals with IUGR predisposes to the development of oxidative stress and postnatal metabolic disorders (Che *et al*., 2015). The aim of the research was to study the dependence of the activity of antioxidant enzymes in the blood of newborn calves with IUGR on their supply with selenium, copper, zinc and magnesium during the intrauterine period. Fifty-three calves of red-mottled breed were examined within 24 hours after birth: 28 with IUGR, and 25 with normal physiological course of pregnancy in the mothers (control group, CG). Criteria for underdevelopment of embryos and fetuses were based on the coccyx- parietal size: (a) on 38th-45th days after insemination and conception, less than 16 mm and the diameter of the body less than 9 mm; at the age of 60- 65 days, less than 45 mm and 16 mm, respectively. On 110th-115th days, IUGR was defined as the diameter of the uterine horn less than 15 cm and the placenta less than 17 mm (Nezhdanov *et al*., 2014). In the blood of calves, the concentration of malonic dialdehyde (MDA) and the activity of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) were determined with a UV-1700 spectrophotometer (Shimadzu, Japan), using the relevant methods described in the literature. The concentration of selenium, copper, zinc, magnesium in the tail hair of calves was determined with an atomic-absorption spectrophotometer AA6300 (Shimadzu). Results were presented as means +/- standard deviation. The reliability of the differences between the groups was determined by comparing the medians of the samples using the non-parametric Wilcoxon test. Correlations between the indicators were detected using Spearman's nonparametric test. It was found that in the hair of IUGR calves the content of selenium was reduced by 26.4% ($P<0.001$), copper by 28.3% ($P<0.001$), zinc by 10.7% ($P<0.001$), magnesium by 9.4% ($P<0.001$), respectively, compared to CG. An increase in the concentration of MDA in the blood of IUGR-calves by 26.8% ($P<0.001$) compared to CG indicated the oxidative stress progression. It is known that during the last 10-15% of the gestation period, the activity of antioxidant enzymes in the fetal tissues increases by 150-200% (Frank and Sosenko, 1987). Since the concentrations of chemical elements in the tail brush hair of newborn calves reflect its accumulation during the last 3 months of intrauterine development, it is obvious that the maturation of the antioxidant system in fetus with IUGR occurs with the deficiency of selenium, copper, zinc and magnesium. Significant correlations were found between the content of copper in the hair and the activity of SOD in the blood ($r=+0.55$, $P<0.05$) and between the content of selenium in the hair and the activity of GPx in the blood ($r=+0.84$, $P<0.01$). Correlations between CAT activity in the blood and the content of trace elements in the calves' hair were not statistically significant. We assume that oxidative stress in newborn IUGR calves is associated with intrauterine deficiency of selenium and copper.

040 Gametes Biology, Embryology and Fetal Development

Lipid profile during bovine initial development unraveled by sensitive MRM approach

C.B. Lima*[1,2], M.P. Milazzotto[1,2], C.R. Ferreira[3], G.R. Cooks[3]

[1]Universidade de São Paulo, São Paulo, SP, Brasil; [2]Universidade Federal do ABC, Santo André, SP, Brasil; [3]Purdue University, West Lafayette, IN, USA.

Spatial and temporal changes in lipidome play fundamental biological roles throughout mammalian initial development, since lipids are involved in key metabolic processes. Recently, MS based techniques have been used to assess the lipid content of gametes and embryos but the lipidome coverage is still limited to a few dozen lipids. Thus, we propose a novel analytical strategy referred as *tailored MRM lipid profiling* to allow the sensitive screening of entire lipid classes. Here, we reported the lipid changes observed throughout preimplantation bovine development using this method. The transitions connecting the precursor ion and a typical fragment for each lipid class (multiple reaction monitoring – MRM) were organized for 10 lipid subclasses listed in the LipidMaps database (Phosphatidylcholine [PC], sphingomyelin [SM], phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, acyl-carnitine, cholesteryl ester, free fatty acids and triacylglycerol). Around 15,000 entries were combined as constitutional isomers, and the resulting 1,588 MRMs were used to screen pooled lipid extracts (representative samples) of the following experimental groups: Single bovine oocytes (immature [IO] and mature [MO]), embryos at 3 different stages (2 cells [2C], 8-16 cells [8C] and blastocysts [BX]). Screening was performed by flow-injection into a triple quadrupole mass spectrometer equipped with an electrospray ionization source. Only transitions (n=389) yielding ion intensity higher than a blank sample (pure solvent) were selected to screen individual oocytes and embryos obtained from at least 3 replicates of each group. Data was processed using an in house built script to obtain the absolute ion intensity and then normalized by the total ion count of each sample. Values of relative ion intensity were submitted to univariate (ANOVA) and multivariate analysis (PCA). Phospholipids such as PC and SM, and many cholesteryl ester ions presented higher relative intensity in IO group compared to MO, indicating the IO tendency to accumulate lipids during pre-maturation stage to form membranes and produce energy during the rapid cell division after oocyte fertilization. The MO group presented significantly higher intensity of free fatty acids such as palmitic (16:0), stearic (18:0) and oleic (18:1) that may be used through the β-oxidation to produce energy and boost maturation progression. When embryos were evaluated by PCA, the first principal component explained 81.8% of the variance in the dataset. Lipid relative amounts were similar between 2C and 8C groups, but distinct from BX. A total of 331 lipids were significant ($P<0.05$) by ANOVA, of which 306 showed increased higher relative intensity in blastocyst stage. The dynamic change from 2C/8C to BX can be related to embryonic genome activation that increases the production of macromolecules to sustain preimplantation development and initial cell fate differentiation which is clear at the blastocyst stage. We conclude that the tailored MRM-profile method allows comprehensive lipidomics assessment of stage-specific lipid profile of bovine oocytes and *in vitro* produced embryos, paving the way for monitoring metabolic impairments associated to lipid metabolism in cattle FAPESP #2015/03381-0 and Capes.

041 Gametes Biology, Embryology and Fetal Development

Addition of pregnancy-associated plasma protein-A (PAPP-A) during *in vitro* maturation did not impact on lipid content of *in vitro*-matured bovine oocytes

T.T. Dellaqua*[1], E.M. Razza[2], V.B. Guilherme[1], A.B. Giroto[3], S. Neves[2], P.H. Santos[2], M.J. Sudano[4], D.B. Müller[4], A.C.S. Castilho[3]

[1]Universidade Estadual Paulista, Assis, São Paulo, Brazil; [2]Universidade Estadual Paulista, Botucatu, São Paulo, Brazil; [3]Universidade do Oeste Paulista, Presidente Prudente, São Paulo, Brazil; [4]Universidade Federal do Pampa, Uruguaiana, Rio Grande do Sul, Brazil.

Insulin-like growth factor (IGF) participates in lipid metabolism and adiposity regulation by the increasing of lipids β-oxidation. The IGF bioavailability is regulated by pregnancy-associated plasma protein-A (PAPP-A), which is able to disrupt IGFs of IGF binding protein (IGFPBs) increasing availabiltiy of free IGF. Recent findings showed that addition of PAPP-A during *in vitro* maturation increases free IGF and impacts on the expression of genes related to lipid metabolism in bovine blastocysts. In this context, the aim of the present work was to investigate the effects of PAPP-A during IVM of cumulus-oocyte complexes (COCs) on lipid content of bovine oocytes. For this, COCs from a local abattoir were submitted to IVM for 24h with TCM199 in presence or absence of 100 ng/mL PAPP-A, as well as of bovine fetal serum (BFS). Therefore, COCs were matured in four different media: bovine serum albumin (BSA), BSA+PAPP-A, BFS or BFS+PAPP-A. Thereafter, matured and denuded oocytes (n=60 oocytes/experimental group) were submitted to semi-quantitative lipid assay with Sudan-Black B dye. Samples were fixed in a 4% paraformaldehyde solution in PBS free of calcium and magnesium, and transferred to 50% ethanol before staining in 1% Sudan-black B (w/v) in 70% ethanol for 2 min. Samples were mounted in glycerol and examined under a light microscope at 400x magnification. The relative lipid content was estimated in Image J 1.41 software (Wayne Rasband, National Institutes of Health, Bethesda, MD). Images of oocytes were converted to a gray-scale and the gray intensity per area was calculated. The effect of PAPP-A was tested by ANOVA using JMP software (SAS Institute Cary, NC). Differences were considered significant when $P \leq 0.05$. The results showed that addition of PAPP-A during oocyte *in vitro* maturation did not alter lipid content (arbitrary units/μm^2) in the presence or absence of bovine fetal serum ($P > 0.05$; BSA: 9.48±1.56; BSA+PAPP-A: 8.96±0.6; BFS:8.66±1.11 and BFS+PAPP-A: 9.46±1.46). In conclusion, PAPP-A added during oocyte *in vitro* maturation is not able to reduce lipid content of matured oocytes. However, this finding does not exclude the possibility that PAPP-A treatment may impact on lipid metabolism at a later stage of bovine embryo development. The authors are grateful to São Paulo Research Foundation (FAPESP; grant #2013/11480-3, #2017/19679-4).

042 Gametes Biology, Embryology and Fetal Development

An investigation into the factors affecting milk pregnancy associated glycoproteins in seasonal-calving pasture-based dairy cows

R.C. Doyle*[1,2], M.M. Herlihy[1], M.C. Lucy[2], S.T. Butler[1]

[1]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland; [2]Division of Animal Sciences, University of Missouri, Columbia, Missouri, USA.

Early pregnancy diagnosis through testing for pregnancy associated glycoprotein (PAG) in milk has the potential to be a useful management tool in seasonal calving systems of milk production. The objective was to examine the effects of week of pregnancy (weeks 5 to 21 post-insemination), fertility index (FI; low <85, medium 85-119 & high ≥120), parity (1, 2, 3+), and interactions between week post insemination and both parity and FI on milk PAG S-N values. Milk PAG S-N values were determined weekly from weeks 5 to 21 post-insemination using the IDEXX Milk Pregnancy Test (IDEXX, USA). Out of the 367 cows enrolled in the study, only cows that conceived to first artificial insemination and maintained pregnancy were retained, resulting in in 2351 observations from 169 cows (n= 53, 76 & 37 for parity 1, 2, and 3+, respectively) available for statistical analysis. PAG S-N values were not normally distributed, an appropriate Box-Cox transformation was identified, and the transformed PAG S-N values were analysed using generalized linear mixed-model (presented as back-transformed LS Means and 95% CI). Week post-insemination (P<0.0001), parity (P 0.065), parity x week post-insemination (P < 0.013) and FI x week post-insemination (P < 0.0028) were all significantly associated with milk PAG S-N values. Peak PAG S-N values were observed on week 5 post-insemination (1.38 [1.25, 1.51]), nadir values on week 9 (0.701 [0.63, 0.77]), followed by recovery thereafter. There was a trend (P = 0.065) for declining milk PAG S-N values with increasing parity number (1.32 [1.23, 1.41], 1.25 [1.17, 1.33] and 1.15 [1.03, 1.27] for parity 1, 2, and 3+, respectively). The interaction between FI and week post-insemination (P = 0.0028) occurred because cows with high FI had a greater PAG S-N values at weeks 5 (1.73 [1.52, 1.96]) and 6 (1.48 [1.33, 1.63]) post-insemination compared with cows with medium FI (1.14 [0.94, 1.36] and 1.32 [1.18, 1.48], respectively) and low FI (1.27 [1.09, 1.48] and 1.12 [0.98, 1.27], respectively), but differences were not detected thereafter. An interaction between parity and week post-insemination was also observed. Parity 1 cows tended to have greater mean nadir PAG S-N values (0.79, [0.67, 0.92]) at week 8 post-insemination compared with parity 2 (0.69 [0.59, 0.79]) and parity 3+ (0.64 [0.49, 0.79]). Most of the differences in milk PAG concentrations were observed before week 12 post-insemination. The temporal variation in milk PAG S-N values in seasonal calving pasture-based cows are consistent with previous reports from high yielding cows fed a total mixed ration. Dam genetic merit for fertility traits may also contribute to the variation in the PAG signal during weeks 5 to 6 post-insemination.

043 Gametes Biology, Embryology and Fetal Development

Distinct expression profile of microRNAs in maternal serum indicates early pregnancy status of cows

S. Gebremedhn*, D. Salilew-wondim, M. Hoelker, E. Held-Hoelker, C. Neuhoff, E. Tholen, K. Schellander, D. Tesfaye

Department of Animal Breeding and Husbandry, Institute of Animal Sciences, University of Bonn, Bonn, Germany.

In mammals, pregnancy establishment is an evolutionarily conserved and a complex physiological process, which begins with the formation of a zygote in the oviduct. Following the continuous cleavages, the embryo enters the uterine cavity and undergoes rapid changes in size and shape. At this stage, the release of conceptus-derived IFN-tau acts on the maternal endometrium and prevents the regression of corpus luteum and maintains the pregnancy. In modern dairy farms, it is important to confirm the pregnancy status of breeding cows at the very earliest day post-insemination (DPI), so as to rebreed the non-pregnant cows and increase the reproductive efficiency. The currently available pregnancy detection tools have limitations to determine the pregnancy status of cows early and accurately, which resulted in a decline in farm profitability. Thus, development of alternative pregnancy detection tools, which can accurately predict the early pregnancy status of cows, is of relevance. Mature microRNAs (miRNAs) are known to be present in the maternal circulation and have been associated with various pregnancy-related disorders. Here, we determined the expression profile of circulatory miRNAs in serum samples of pregnant and non-pregnant dairy cows, with the aim to identify circulating miRNAs, which can be used as indicators of early pregnancy. For this, lactating Holstein-Friesian cows were estrous synchronized and inseminated with frozen semen. Blood samples were collected 19 and 24 DPI. Following this, ultrasonography was used to determine the pregnancy status of cows 35 days later, and accordingly serum samples were retrospectively categorized. Total RNA enriched with miRNAs was isolated from triplicate pools (4 animals/pool) of serum samples from each category and subjected to cDNA synthesis. The expression signature of circulatory miRNAs was analyzed using a PCR array containing primers of 748 mature miRNAs. Results showed that a total of 302 and 316 miRNAs were detected in the circulation of day 19 pregnant and non-pregnant cows, respectively. Similarly, 356 and 325 miRNAs were detected in day 24 pregnant and non-pregnant cows, respectively. Principal component analysis separated cows according to the pregnancy status both at 19 and 24 DPI. Moreover, 8 and 23 miRNAs were found to be differentially expressed in the serum of pregnant cows at day 19 and 24, respectively. One miRNA (miR-433) and four miRNAs (miR-487b, miR-495-3p, miR-376b-3p, and miR-323a-3p), which are homologous to the human pregnancy-associated C14MC miRNA cluster were among the differentially expressed miRNAs at day 19 and 24 of pregnant cows, respectively. MiRNAs differentially expressed in the serum of pregnant cows were predicted to be involved in adherens junction and ECM-interaction pathways. In conclusion, the expression profile of miRNAs in maternal circulation could be associated with the pregnancy status of cows and be indicators of early pregnancy.

044 Gametes Biology, Embryology and Fetal Development

The kinetics of the first cleavages impacts on the regulation and levels of histone acetylation of bovine blastocysts

J. Ispada[1,2], C.B. Lima[1,2], O.L.R. Santos[2], A.M. Fonseca-Junior*[2], E.C. Santos[2], P.K. Fontes[3], M.F.G. Nogueira[3], M.P. Milazzotto[1,2]

[1]Universidade de São Paulo, USP, São Paulo, SP, Brazil; [2]Universidade Federal do ABC, Santo André, SP, Brazil; [3]Universidade Estadual de São Paulo, UNESP, Assis, SP, Brazil.

In bovine embryos, distinct kinetics during the first cleavages is associated with blastocysts with different metabolic activity, gene expression and DNA methylation levels. However, there are no previous reports of alterations in histone regulation. The present work investigated the presence of acetylation on lysine 9 of histone H3 (H3K9ac), one of the most abundant histone alterations in mammal cells. Also, this work evaluated the mRNA levels of H3F3a, H3F3b, HDAC2 and DNMT1, the first two encoding the same histone variant protein associated with active transcription regions, and the last two encoding enzymes that integrate a transcriptional repressor complex. Embryos were produced in vitro with sexed semen using standard protocols and classified at 40 hours post insemination (hpi) as Fast (F) (4 or more cells) and Slow (S) (2 cells). Embryos from both groups were collected at 40hpi (Cleavage [CL] - FCL; SCL), 96hpi (Embryonic genome activation [EGA] - FEGA; SEGA) and 168hpi (Blastocyst [BL] - FBL; SBL) for evaluation of H3K9ac using immunofluorescence (minimum of 5 and maximum of 10 embryos/group, from 3 replicates) and transcript quantification (12 blastocysts/group from 4 replicates) by RT-PCR. The fluorescence intensity from each nucleus was quantified using ImageJ (Total number of nucleus analyzed per stage: 63 CL, 184 EGA and 1192 BL), normalized by the nucleus area and compared by non-parametric Mann-Whitney (kinetics) or Kruskal-Wallis test (stage). For RT-PCR, PPIA and GAPDH were used as endogenous controls for ΔCt calculation and data were submitted to Mann-Whitney test. H3K9ac fluorescence intensity was lower in slow compared to fast embryos at CL and EGA stages (FCL 164.6±10.1 A.U. vs SCL 96.5±11.7A.U.; FEGA 119.1±5.6A.U. vs SEGA 71.1±4.9A.U.; $P<0.0001$) but was higher at BL stage (FBL 69.7±2.3 A.U. vs SBL 274.5±16.3 A.U.; $P<0.0001$). It is important to highlight that acetylation of H3K9 decreased through development ($P<0.0001$) for fast embryos, but increased from EGA to BL stage in slow embryos ($P<0.0001$). Regarding gene expression analysis, no difference was found between mRNA levels of H3F3b, only of H3F3a ($P=0.06$), which was overexpressed in SBL than FBL. Also, there was no difference for HDAC2 mRNA levels, only DNMT1 ($P=0.05$) was underexpressed in SBL. Transcription analysis suggests that there was a replacement of histone 3 with one variant associated with more active transcription regions in SBL and that the HDAC2-DNMT1 complex might be less active in SBL, leading to an increase in transcriptional activity in this group. Based on the results for H3K9ac and gene expression of H3F3a and DNMT1, it is possible to expect that FBL show lower transcription activity than SBL. The present study corroborates previous reports from our group in which FBL presented increased levels of DNA methylation, suggesting that this group has lower transcription activity than SBL. The present data also indicate that the difference between FBL and SBL originates in earlier stages and is associated with distinct status of H3K9 acetylation during preimplantation development. However, it is worth investigating if other variations in epigenetic factors are associated with alterations in gene expression. FAPESP #15/03381-0 and Capes.

045 Gametes Biology, Embryology and Fetal Development

Gene expression related to meiosis resumption after associated use of NPPC and rhFSH during *in vitro* prematuration of bovine *cumulus*-oocyte complexes

E.M. Pioltine*[1], A.G. Sawa[2], A.M. Almeida Junior[2], M. Pronunciate[1], F.F. Franchi[1], M.F.G. Nogueira[1,2]

[1]Universidade Estadual Paulista, Departamento de Farmacologia, Botucatu, São Paulo, Brazil; [2]Universidade Estadual Paulista, Departamento de Ciências Biológicas, Assis, São Paulo, Brazil.

Studies have shown that natriuretic peptide C (NPPC) and its type 2-receptor (NPR2) are essential for the maintenance of oocyte meiotic arrest. FSH is widely used to stimulate *cumulus* cell expansion and meiosis progression. Since the active NPPC-NPR2 system can delay spontaneous resumption of meiosis *in vitro* and FSH possesses an antagonistic action, the combined use of those drugs is avoided when designing systems for in vitro maturation (IVM) or pre-IVM. The objective of this study was to evaluate the effect of NPPC, associated or not to rhFSH in a 6 h pre-IVM system on the mRNA relative abundance of genes associated with meiosis resumption in bovine oocytes. *Cumulus*-oocyte complexes (COCs) were matured according to the following groups: I) Experimental control: 6 h pre-IVM with basic medium (M199, BSA, pyruvate and amikacin); II) Pre-IVM-NPPC: 6 h pre-MIV with basic medium plus NPPC (100 nM); III) Pre-IVM-FSH: 6 h pre-IVM with basic medium plus rhFSH (0.1 IU/mL); IV) Pre-IVM-NPPC/FSH: 6 h pre-IVM with basic medium plus NPPC and rhFSH. COCs were collected at 9 h and 15 h of culture (pre-IVM time plus IVM time; *i.e.*, COCs from groups I, II, III and IV were collected after 3 h and 9 h of IVM). Five replicates were performed with 20 COCs/group and the oocytes were separated from their *cumulus* cells by successive pipetting. Total RNA was extracted by RNeasy® kit (Qiagen) and reverse transcribed using High Capacity Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). For the analysis of the gene expression we used the integrated high performance microfluidic system Biomark® HD, (Fluidigm, San Francisco, CA, USA) with Applied Biosystems™ TaqMan® Assays, specific for *Bos taurus*. The expression of target genes was normalized by geometric mean of three reference genes (*B2M, PPIA* and *SDHA*). The effect of treatments was tested by one way-ANOVA and the means were compared with Tukey Test. Differences were considered significant when $P<0.05$. From ten genes analyzed (*ADCY3, ADCY6, ADCY9, BDNF, IMPDH1, IMPDH2, MAPK1, NOS2, NOS3* and *PDE5A*), a difference in transcript relative abundance was found for two of them: *NOS3* and *ADCY9* (respectively at 9 h and 15 h). At 9 h IVM, groups II (NPPC) and IV (NPPC/FSH) had decreased mRNA content in relation to group I (Control). At 15 h IVM, groups III (FSH) and IV (NPPC/FSH) were up regulated when compared with group I (Control). These results suggest that after 9 h and 15 h of maturation, there was not a difference in mRNA relative abundance in the analyzed genes related to resumption of meiosis in bovine oocytes submitted to antagonistic action treatments (separately or in a combined way). Funding: grant #2012/50533-2, São Paulo Research Foundation (FAPESP). *In vitro* maturation; Oocyte meiotic arrest; NPPC; FSH; Gene expression; *Bos taurus*.

046 Gametes Biology, Embryology and Fetal Development

Dynamic changes in miRNAs during early embryonic development in bovine: From oocytes to *in vitro* produced blastocysts

R. Mazzarella, M. Del Collado, G. Andrade, F. Perecin, F. Meirelles, J. Silveira*

University of São Paulo, Faculty of Animal Sciences and Food Engineering, Pirassununga, São Paulo, Brazil.

Upon fertilization maternal transcripts are gradually replaced by embryonic mRNAs until the major embryonic genome activation (EGA), which occurs around the 8-16 cells stage. Short non-coding RNAs, microRNAs (miRNAs), can regulate gene expression through post transcriptional mechanisms, and can play a critical role regulating transcripts such as the maternal mRNA during the maternal-to-embryonic transition as well as after the major EGA. The aim of this study was to determine the dynamics of miRNAs during early bovine embryonic development *in vitro*. Slaughterhouse ovaries were used to obtain oocytes from 3-6 mm follicles while bovine embryos were produced by *in vitro* fertilization (IVF). Samples were collected as denuded mature oocytes as well as day 2 (4 cells), day 3 (8-16 cells), and morulae and blastocysts following IVF. Mature oocytes were fertilized with semen from one bull. *In vitro* culture (IVC) was performed at 38.5°C, 5% CO2 and 5% O2. Samples were grouped in three pools of 20 denuded oocytes or 10 embryos collected on day 2 (D2), containing 4 cells, 10 embryos collected on day 3 (D3), containing between 8 and 16 cells, and 10 morulae and 10 blastocysts. Oocytes and embryos were collected from 6 different embryo production routines and homogeneously distributed among pools. Total RNA quality was determined by Nanodrop. Reverse transcription was performed with 100 ng of total RNA utilizing the miScript PCR System kit (Qiagen). Real- time PCR analysis of the miRNAs was performed with a custom miRNA profiler plate. Data were normalized by the geometric mean of 2 endogenous small RNAs (RNU43 snoRNA and bta-miR-99b). The relative levels ΔCt were analyzed by ANOVA followed by Tukey-Kramer HSD test comparing pools as experimental unit. A total of 157 miRNAs were commonly identified from oocyte to blastocyst stage. The total number of miRNAs identified in each stage was 277 in oocytes, 255 in D2, 278 in D3, 265 in morulae and 285 in blastocyst. The total number of miRNAs identified as unique for each stage were seven for oocytes, four for D2, six for D3, none for morulae and 13 for blastocyst. A total of 25 miRNAs were differentially expressed comparing from oocyte throughout blastocyst stage ($P<0.045$ to $P<0.01$). Additionally, a total of 36 miRNAs were differently expressed comparing oocytes, D2 and D3 ($P<0.046$ to $P<0.01$). Bioinformatics analysis indicated similar pathways regulated by the different miRNAs such as PI3K-AKT, ErbB signaling and signaling pathways regulating pluripotency and stem cells. However, pathways such as RNA degradation, oocyte meiosis as well as biosynthesis of amino acids and cell cycle were enriched when we compared oocytes throughout blastocysts and oocytes throughout D3 embryos, respectively. Thus, our analysis demonstrated that miRNA levels are dynamic during early embryo development, suggesting they may play an important role in modulating cell differentiation and metabolism. The results of this project will allow a better understanding of pathways regulated by miRNAs during early embryonic development, with important implications for the assisted reproduction industries in cattle and humans. Financial support: FAPESP 2014/22887-0, 2014/03281-3, 2015/21674-5, 2013/08135-2 and 2012/50533-2.

047 Gametes Biology, Embryology and Fetal Development

Interferon-tau is induced in bovine Day-4 embryos by oviduct epithelium, which generates an anti-inflammatory response in immune cells

A. Miyamoto*[1], A.K. Talukder[1,2], M.B. Rashid[1,3], M.S. Yousef[1,4], K. Kusama[5], M. Shimada[6], S.S. Suarez[7], K. Imakawa[5]

[1]Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan; [2]Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh; [3]Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh; [4]Assiut University, Assiut, Egypt; [5]The University of Tokyo, Ibaraki, Japan, [6]Hiroshima University, Higashi-Hiroshima, Japan; [7]Cornell University, Ithaca, NY, USA.

Early cleavage-stage bovine embryos express *MHC I* transcript and thus they could be detected as foreign by the oviduct immune system. The molecular mechanism by which bovine embryos avoid maternal immune rejection has not been well characterized. Here, we used an *in vitro* model to investigate the effect of embryos on immune responses in the oviduct. First, zygotes were cultured with or without bovine oviduct epithelial cells (BOECs) for 4 days, when embryos had reached the 16-cell stage. At that time, interferon-tau (IFNT) was immunohistochemically identified in embryos co-cultured with BOECs, but not in embryos cultured alone (n=19 each). Co-culture of embryos (25-30/well, n=6) with BOECs induced an anti-inflammatory response in BOECs *via* suppression of *NFkBIA* and *NFkB2* (inflammatory response mediators; $P<0.01$), and stimulation of *PTGES* (enzyme involved in PGE2 synthesis; $P<0.05$), as well as secretion of PGE2 (immunosuppressive prostaglandin; $P<0.05$). Next, peripheral blood mononuclear cells (PBMCs) were incubated for 24 h either in media from embryo cultures or from co-cultures of embryos with BOECs. The medium from embryo cultures did not modulate PBMCs gene expression; whereas the embryo-BOEC co-culture medium increased interferon (IFN)-stimulated genes (*ISGs*: *ISG15*, *OAS1*, *MX2*), *STAT1* (IFN signaling factor), *PTGES* and *TGFB1* (anti-inflammatory cytokine) but suppressed *IL17* (pro-inflammatory cytokine) expression in PBMCs ($P<0.05$). Both IFNT-treated BOEC culture medium and IFNT-supplemented fresh medium without BOEC, modulated PBMCs gene expression, similar to the effects of embryo-BOEC co-culture medium. Further, specific antibody to IFNT neutralized the effect of embryo-BOEC co-culture medium. Our findings indicate that BOECs stimulate embryos to produce IFNT, which then acts on immune cells to promote an anti-inflammatory response in the oviduct. This study provides new insight into the molecular mechanism by which a semi-allogenic embryo avoids immune rejection in the bovine oviduct. This study was supported by Grant-in Aid for Scientific Research (16H05013) of Japan Society for the Promotion of Science (JSPS).

048 Gametes Biology, Embryology and Fetal Development

Seasonal influence on spermatic parameters and testosterone levels of Santa Inês rams

C.F. Moya-Araujo*[1], M. Piaggentini[2], D.C. Silva[3], G.H.M. Araujo[4], L. Bicudo[5], T.E. Cruz[5], L.C. Salgado[5], E. Oba[5]

[1]Center-western State University - UNICENTRO, Guarapuava, PR, Brazil; [2]Eduvale, Avaré, SP, Brazil; [3]Autonomous veterinary, Avaré, SP, Brazil; [4]Goias Federal University - UFG, Jataí, GO, Brazil; [5]FMVZ/UNESP, Botucatu, SP, Brazil.

The time of the year elected to perform the reproductive management of an ovine breeding farm must be based on a series of factors, including the weather and the nutrition and reproductive capacity of the male. Testosterone concentrations as well as seminal characteristics are influenced by the photoperiod. In Brazil, the ovine reproductive season is concentrated around October. The present study aimed to evaluate the seasonal effects on sperm parameters and on the serum concentration of testosterone of Santa Inês rams. We used 10 males older than 10 months, housed in individual pens in an intensive system, fed a balanced ration of hay and mineral supplementation. An andrological examination was performed before the animals were selected and at the end of each season (Spring, Summer, Autumn and Winter). Semen was obtained by electroejaculation. Evaluated parameters were volume (mL), mass movement (1 to 5), motility (%), vigor (1 a 5), sperm concentration, major defects (%), minor defects (%), scrotal circumference (cm) and testosterone serum concentration (ng/dL). The statistical analysis was performed using ANOVA with 5% of significance, followed by Tukey test when necessary. The mean and SEM of the obtained results were: Spring - volume = 0.83 ± 0.09^{b}, mass movement = 4.67 ± 0.21^{a}, motility = 86.67 ± 2.11^{ab}, vigor = 4.65 ± 0.21^{ab}, spermatic concentration = $1.54 \pm 0.61x10^{9a}$, major defects = 2.50 ± 0.62^{b}, minor defects = 7.67 ± 1.84^{a}, scrotal circumference = 27.75 ± 1.07^{a} and testosterone = 1.11 ± 0.26^{b}; Summer - volume = 0.97 ± 0.16^{ab}, mass movement = 4.25 ± 0.17^{a}, motility = 82.50 ± 1.71^{b}, vigor = 4.17 ± 0.17^{b}, spermatic concentration = $0.70 \pm 0.11x10^{9b}$, major defects = 7.50 ± 2.32^{a}, minor defects = 6.17 ± 1.78^{a}, scrotal circumference = 28.67 ± 1.66^{a} e testosterone = 1.15 ± 0.24^{b}; Autumn - volume = 1.36 ± 0.15^{a}, mass movement = 4.73 ± 0.14^{a}, motility = 88.18 ± 1.22^{a}, vigor = 4.73 ± 0.14^{ab}, spermatic concentration = $1.38 \pm 0.14x10^{9a}$, major defects = 6.86 ± 1.39^{ab}, minor defects = 5.21 ± 0.87^{a}, scrotal circumference = 31.36 ± 0.90^{a} e testosterone = 3.76 ± 0.64^{a}; and Winter - volume = 1.20 ± 0.13^{ab}, mass movement = 4.92 ± 0.08^{a}, motility = 89.17 ± 0.84^{a}, vigor = 4.92 ± 0.08^{a}, spermatic concentration = $1.66 \pm 0.19x10^{9a}$, major defects = 5.50 ± 0.76^{ab}, minor defects = 5.33 ± 0.67^{a}, scrotal circumference = 30.42 ± 1.09^{a} and testosterone = 1.79 ± 0.40^{ab}. We conclude that season of the year affected spermatic parameters and serum concentration of testosterone in Santa Inês rams under the experimental conditions. Financial support: FAPESP (no. 2011/51503-7).

049 Gametes Biology, Embryology and Fetal Development

Hormonal metabolic aspects of embryonic mortality of cows and the effectiveness of its prevention

A.G. Nezhdanov*[1], V.A. Safonov[2], S.V. Shabunin[1], V.I. Mikhalev[1], K.A. Lobodin[3],

[1]All-Russian Research Veterinary Institute of Pathology, Pharmacology and Therapy, Voronezh, Russian Federation; [2]Vernadsky Institute of Geochemistry and Analytical Chemistry, Moscow, Russian Federation; [3]Voronezh State Agricultural University N.A. Emperor Peter the Great, Voronezh, Russian Federation.

Embryonic mortality and high frequency of its manifestation among lactating cows is one of the reasons for their fertility impairment and large economic losses in dairy cattle breeding. It is assumed that the determinants of this pathology are metabolic imbalance, hypoprogesteronemia and disorders of the immune- trophic interrelations in the mother-embryo system. The objective of this study was to assess the hormonal, antioxidant and endotoxic status of cows during the physiological formation of the embryo, during its development, retardation and death, as well as the effectiveness of a series of biologically active medications to reduce embryonic losses. The research was performed on 196 black-motley Holstein cows with an average annual milk productivity of 6.5-7.5 thousand kg. Diagnosis of pregnancy, embryo state and its death was carried out on 19-23, 28-32 and 38-45 days after insemination by detecting the progesterone concentration level in serum and by means of transrectal ultrasound scan of reproductive organs. The metabolic status was assessed according to the content of selenium, vitamin E, malonic dialdehyde (MDA), medium molecular peptides (MMP), and glutathione peroxidase (GPO) catalase activity in the blood serum. To prevent the death of embryos such additives as progesterone, gonadotropin PMS (pregnant mare serum), selenium and interferon-tau were used, which were assigned to the cows within the first days after insemination. The embryonic death among intact animals was registered in 29% of cases and was associated with a deficiency of progesterone, estradiol- 17ß, selenium, with a functional disorder of the antioxidant defense system and an increased level of endogenous intoxication. The concentration of progesterone in their blood was lower by 30.6-43.3% ($P<0.05$-0.01), estradiol by 31.0-46.1% ($P<0.05$-0.01), selenium by 26.3-33.2% ($P<0.05$), vitamin E by 31.2% ($P<0.01$), catalase by 27.3% -51.2% ($P<0.001$), the MDA content by 38.6-58.5% ($P<0.01$), the MMP - by 23.9-34.7% ($P<0.05$) and the endogenous intoxication index by 28.8-30.0% ($P<0.001$). Pharmacological control of the hormonal and metabolic status of inseminated cows by prescribing the above mentioned medications, reduced the intrauterine death of the embryo by 12.5-16.7% and increased the effectiveness of insemination by 1.5-1.8 times. The most efficient results were registered in the group of complex use of gonadotropin PMS and selenium. The size of developing embryos among the cows from the experimental groups exceeded the one among the control animals by 35-40% ($P<0.01$).

050 Gametes Biology, Embryology and Fetal Development

Protective effects of cysteamine on bovine oocytes matured *in vitro* with eicosapentaenoic acid (EPA): impact on early embryo development

N. Nikoloff*, A. Campagna, A. Carranza, J.M. Anchordoquy, J.P. Anchordoquy, M. Fabra, M. Pascua, A. Seoane, C. Furnus

IGEVET, Instituto de Genética Veterinaria "Ing. Fernando N Dulout" (UNLP-CONICET-CONICET LA PLATA), Facultad de Ciencias Veterinarias - UNLP, La Plata, Buenos Aires, Argentina.

Data on fatty acid composition and their uptake and utilization by oocytes and embryos are relevant for culture *in vitro*, cryopreservation practices, and embryo quality improvement. Despite several studies *in vitro* suggesting enhanced embryo development with polyunsaturated fatty acids supplementation, results are not consistent and it would be important to determine which fatty acid is the most beneficial for embryo quality and survival (Santos *et al*., 2008). *In vitro*, embryos are sensitive to lipids effects unless adequate antioxidant protection is provided (Reis *et al*., 2003). The aim of this study was to determine the effect of eicosanopentaenoic acid (EPA; n-3, 20:5) alone or with cysteamine (CYS) on bovine oocyte during *in vitro* maturation (IVM). For this purpose, bovine cumulus-oocyte complexes (COC) from ovaries obtained in a slaughterhouse were matured *in vitro in* TCM- 199 supplemented with 0.1% essentially fatty acid–free BSA, 1 µg/mL FSH, 1 µg/mL estradiol 17ß at 39°C with 5% CO_2 in humidified air. COCs were matured 24 h in IVM media: (1) alone (Control); (2) supplemented with 1 nM, 10 nM and 100 nM EPA and, (3) with EPA + Cysteamine 100 µM. Oocyte intracellular ROS level with H2DCFDA, cumulus expansion area with digitalizing images, nuclear maturation with Hoechst 33342, and embryo developmental capacity were evaluated. Data were analyzed using Statgraphics Plus 5.1 software. The results demonstrated that: a) ROS levels were significantly higher in oocytes matured with 1mM and 100 mM EPA compared with Control ($P<0.05$). The presence of CYS diminished ROS levels induced by EPA ($P<0.05$); b) Cumulus expansion area was similar in COC matured with EPA and EPA+CYS ($P>0.05$); c) EPA (100 nM) resulted in a decrease of 10.95% in nuclear maturation rate ($P<0.05$). However, addition of CYS to EPA supplemented media attenuated this effect; and d) embryo development was not altered with EPA, but EPA 1nM + CYS increased significantly cleavage and blastocyst rates in relation to Control values ($P<0.05$). In conclusion, EPA supplementation during bovine IVM increased subsequent embryo development only when CYS was also present in IVM medium providing protection against intracellular ROS generated by EPA. These results are relevant for including EPA in *in vitro* embryo production (IVP) media with the purpose to enhance embryo quality for cryopreservation practices.

051 Gametes Biology, Embryology and Fetal Development

Influence of preovulatory estradiol on uterine luminal fluid proteomics around maternal recognition of pregnancy in beef cattle

E. Northrop*[1], J. Rich[1], R. Cushman[2], X. Ge[1], G. Perry[1]

[1]South Dakota State University, Brookings, SD, United States; [2]USDA, ARS, Roman L. Hruska US Meat Animal Research Center, Clay Center, NE, United States.

Proteins within the uterine luminal fluid (ULF) are involved in elongation, recognition of pregnancy, implantation, and placentation, and previous research has established that elevated preovulatory estradiol increased embryo survival and pregnancy rates. However, on d 16 of pregnancy no differences in conceptus recovery rates or IFNT concentrations were detected between animals with elevated or low concentrations of estradiol prebreeding. The present study evaluated the effects of preovulatory estradiol and conceptus presence on the d 16 ULF proteome. Beef cows/heifers (n=29) were synchronized and artificially inseminated (d 0), and grouped into high (highE2) and low (lowE2) preovulatory estradiol based on expression of estrus and circulating estradiol concentrations (d-2 and d0). On d 16, animals were slaughtered and uteri were flushed. Two separate ULF pools were created based on the following groupings: highE2/no conceptus, highE2/conceptus, lowE2/no conceptus, and lowE2/conceptus. Pools were then analyzed using a 2D LC-MS/MS based 8plex iTRAQ quantitative method. Scaffold Q+ was used to quantitate peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95% probability by Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at greater than 99% probability and contained at least 2 identified peptides. The FDR was adjusted using Benjamini-Hochberg procedure to identify significance ($P < 0.05$) based on permutation tests. String database was used to analyze upregulated and downregulated proteins in each grouping. There was a total of 6989 peptides and 1269 proteins identified, and a total of 48 significant differentially expressed proteins between the highE2/conceptus group and the lowE2/conceptus group, 19 of which were upregulated (GOT2, ACAA1, TSTD1, GPLD1, ALDH2) and 29 were downregulated (UTMP, ORM1, ANXA8, ANXA1, FGG) in the highE2/conceptus group. Proteins associated with cell differentiation, single organism process, and regulation of metabolic process were upregulated in highE2/conceptus group. Proteins associated with angiogenesis, regulation of vesicle mediated transport, and positive regulation of transport were downregulated in highE2/conceptus group. A previous study identified 30 unique proteins produced by a short term culture of d 16 concepti, 24 were identified in the current study (20 were upregulated and 4 were downregulated in the highE2/conceptus group). These data provide insight on differences in specific protein abundances in the ULF that may simply reflect developmental processes, but also may contribute to improved conceptus survival among highE2 animals. USDA is an equal opportunity provider and employer.

052 Gametes Biology, Embryology and Fetal Development

Differential expression of LH receptor, LHR mRNA binding protein (LRBP) and bta-miR-222 in the developing bovine ovary

M.P. Chaves*[1], P.H. Santos[2], L.O. Mendes[1], A.C. Souza Castilho[1]

[1]Universidade do Oeste Paulista, Presidente Prudente, São Paulo, Brazil; [2]Universidade Estadual Paulista "Júlio de Mesquita Filho, Assis, São Paulo, Brazil.

Gonadotropins play an essential role during antral follicular development, as well as in late stages of preantral follicle development. Although the luteinizing hormone receptor (LHR) has been detected in preantral follicles of rats, rabbits, and pigs, the expression and localization of LHR in bovine fetal ovaries have not been assessed. Thus, the present study aimed to quantify the expression of LHR in the developing bovine ovary. In addition, mRNA abundance of LHR binding protein (*LRBP*) and levels of bta-miR-222 were also quantified. Twenty bovine fetuses (predominantly *Bos taurus indicus*) were obtained in a local slaughterhouse and divided into four groups (n=5/group) according to gestational age estimated by the crown-rump length (gestational ages: days 60, 90, 120 and 150). Both ovaries were removed and one was destined to immunohistochemical analysis, while the other was submitted to total RNA extraction. The expression of *LHCGR* and *LRBP* was investigated by RT-qPCR using the Sybr Green system and LHR immunolocalization was performed by immunohistochemistry. miRNA extraction was performed using 50 µg of total RNA into *mir*Vana™ miRNA Isolation Kit (Life Technologies®, São Paulo, Brazil) and for reverse transcription and qPCR of target miRNAs, TaqMan® Reverse Transcription Reagents following manufacturer's protocols were utilized. For immunostaining of LHR, slides were incubated with primary antibody (anti-LH receptor, ab96603, Abcam®, Cambridge, UK) and then incubated with biotinylated secondary antibody followed by a VECTASTIN ABC Kit (Vector Laboratories Ltd.). Slides of mature *corpus luteum* were used as positive control. The effect of gestational age was tested by ANOVA and means were compared by Tukey-test. Differences were significant when $P \leq 0.05$. In summary, the *LHCGR* expression was present in the bovine fetal ovary and showed lower mRNA abundance on day 150 compared with day 60 (P=0.04). Nevertheless, the mRNA abundance of *LRBP* presented an opposite pattern, showing higher expression on day 150 (P=0.03). Similar as *LRBP* expression, the abundance of bta-miR-222 was higher on day 150 compared with days 60 or 90 (P=0.02). The LHR protein was immunolocalized in oogonias, primordial, primary and secondary preantral follicles. Moreover, both oocyte and granulosa cells showed LHR immunostaining. Another important finding was stronger immunoreactivity for LHR in the ovarian cortex compared with the medullary region and staining in the ovarian surface epithelium and blood vessels. In conclusion, these findings may suggest that the *LHCGR/LRBP* regulation could be involved in the mechanisms regulating preantral follicle development, especially during establishment of secondary follicles. Furthermore, the present data suggest that lower expression of *LHR* mRNA in bovine fetal ovaries at day 150 could be related to higher expression of *LRBP* and bta-miR-222, as described in granulosa cells during follicular deviation in cattle. The authors are grateful to São Paulo Research Foundation (FAPESP; grant #2013/11480-3).

053 Gametes Biology, Embryology and Fetal Development

Transcripts abundance modulation by AREG and FSH treatments during the *in vitro* maturation of bovine *cumulus*-oocyte complexes

M. Pronunciate*[1], E.M. Pioltine[1], V.B. Guilherme[2], F.F. Franchi[1], P.K. Fontes[1], M.F.G. Nogueira[2,1]

[1]Universidade Estadual Paulista, Botucatu, São Paulo, Brazil; [2]Universidade Estadual Paulista, Assis, São Paulo, Brazil.

The LH surge induces in the ovarian follicle the prompt production of cAMP that, in turn, subsequently stimulates the synthesis of EGF-like peptides: Amphirregulin (AREG) Epirregulin (EREG) and Betacelulin (BTC) in the granulosa cells. They are cleaved, released and bind to their receptor in granulosa and *cumulus* cells (CC), where they act stimulating the expression of critical genes for the CC expansion among other pathways. Moreover, they mimic the *in vivo* LH action when they are added *in vitro* (*i.e.*, they promote nuclear and cytoplasmic maturation). LH action on CC is mediated by EGF-like peptides and previous data suggest that they are superior to the FSH and EGF in *in vitro* maturation (IVM). The aim of this work was to evaluate transcript abundance of a gene set downstream to the LH surge pathway (*e.g.*, FSHR, EGFR, LHCGR *etc*.) in cumulus-oocyte complexes (COCs) after IVM with FSH or AREG. Ovaries were obtained from an abattoir and follicles between 2-8mm of diameter were aspirated. COCs (n=25 COCs/group; 5 replicates) were *in vitro* matured in a medium with BSA and AREG (100 ng/mL) or rhFSH (0.1 IU/mL; Gonal-*f*® 75 IU) or in a control medium [rhFSH (0.1 IU/mL; Gonal-*f*® 75 IU) plus FBS] at 38.5°C and 5% CO_2 humid atmosphere for 15 or 24h. Oocytes were separated from CC by pipetting and vortexing and stored at -80°C. Total RNA was extracted, DNAse treated, reverse transcribed using random primers and submitted to qPCR using the integrated high performance microfluidic system Biomark® HD (Fluidigm). The results were normalized using the geometric mean of *ACTB*, *B2M* and *RPL30* and analyzed by ANOVA or Kruskal-Wallis test using the JMP® software. Significant differences were considered when $P \leq 0.05$. Regarding the expression of the hormone receptors (EGFR, FSHR and LHCGR), there was no difference among groups except for EGFR (higher in FSH than in AREG after 15h of maturation in CC; $P = 0.0397$). From the 96 genes tested (related to thermal stress, apoptosis, cell cycle, cumulus expansion, blastocyst quality prediction and others) 23 were less abundant in CC samples from AREG group when compared to the control or FSH groups (*e.g.*, *BAX, BCL, VNN1, HSP1, HSPA1A* and *FOXO3*), whereas in oocytes two genes were less abundant in the AREG group compared to the control or FSH groups (*EGFR* and *HAS*). We observed a non-overlapping gene expression effect of the two ligands, consistent with the hypothesis of different pathways being activated with AREG or FSH during the IVM. Funding: grant #2012/50533-2, São Paulo Research Foundation (FAPESP).

054 Gametes Biology, Embryology and Fetal Development

Lack of association of neospora caninum infection with late embryo losses in grazing dairy cows

L.E. Quintero Rodriguez*[1], L.M. Campero[2,3], G. More[2,3], G. Dominguez[4], M.C. Venturini[2], R.L. de La Sota[1,3], L.V. Madoz[1,3], M.J. Giuliodori[5]

[1]Cátedra y Servicio de Reproducción Animal, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina; [2]Laboratorio de Inmunoparasitología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina; [3]Comisión Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Buenos Aires, Argentina; [4]Private practice, Argentina; [5]Cátedra de Fisiología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina.

The fertility of high-producing, lactating dairy cows has decreased during the last decades. Pregnancy losses in dairy cows are an important aspect of infertility leading to poor economic performance. Late embryo losses (LEL) are assumed to be of multifactorial origin, with *N. caninum* infection being a potential cause. The objective of this study was to evaluate the association of *N. caninum* infection with LEL in grazing dairy cows. A case-control study was carried out in a commercial dairy herd (32°49´ S, 62°52´ W, Argentina) in which Holstein cows (n=92) were enrolled. Pregnancy was diagnosed by ultrasonography at 30-44 days post-insemination. Lack of heart beats, identification of membrane detachment, and disorganization or echoic floating structures including embryo remnants were used as indicators of LEL (CASE cows). Cows with positive pregnancy diagnosis on the same date of each case were considered as control (CON) and the ratio of case-control was 1. For each case animal, the conceptus was sampled (day 0) with an insemination pistol attached to a 10-mL syringe and transported at 4°C in a 1.5-ml tube. Cows were bled on days 0 and 30 for serological studies. Blood samples were refrigerated at 4°C and transported to the laboratory within 24 h, centrifuged and sera were stored at -20°C until analysis. Sera were tested by indirect fluorescent antibody test (IFAT) for the detection of antibodies against *N. caninum* using a cut-off titer of 1:200 and processed to final titer. The DNA from conceptuses from seropositive cows was extracted using a commercial kit according the manufacturer's recommendations. The DNA samples were analyzed by PCR with the specific primer pair Np6+/Np21+ for *N. caninum*. Chi squared test was used to detect differences between groups and $P<0.05$ was considered as significant. The proportion of seropositive cows to *N. caninum* in one or both sampling points was similar (P=0.599) between CASE (26%, 12/46) and CON (19.6%, 9/46) groups. The proportion of cows showing seroconversion was 6.5% (3/46) and 4.3% (2/46) for CON and CASE groups, respectively (P=0.212). Finally, PCR analysis was negative for *N. caninum* DNA in all the conceptus from CASE cows (n=12). We conclude that *N. caninum* is not associated with LEL in grazing dairy cows from Argentina.

055 Gametes Biology, Embryology and Fetal Development

Serum concentration of sex steroids in down-calving cows as predictors of the respiratory diseases progression among their posterity

V.A. Safonov*[1], A.E. Chernitskiy[2]

[1]Vernadsky Institute of Geochemistry and Analytical Chemistry, Moscow, Russian Federation; [2]All-Russian Research Veterinary Institute of Pathology, Pharmacology and Therapy, Voronezh, Russian Federation.

DoHaD (developmental origins of health and disease) hypothesis proposes that early-life environment has long-term effects on the postnatal ontogenesis (Fukuoka, J Nutr Sci Vitaminol 61:S2–S4, 2015). Of great interest is the study of the role of sex steroids in perinatal and early postnatal development, in order to identify their potential capacity in imprinting/programming phenomena. The aim of the study was to determine the diagnostic value of serum concentration of progesterone, estradiol and dihydroepiandrosterone sulfate (DHEA-C) in down-calving cows to predict the respiratory diseases progression among their posterity. Forty-five cows of red-motley breed with one-fetal pregnancy at gestation period of 248-255 days and calves obtained from them were examined. The concentration of progesterone, estradiol and DHEA-S in the serum of cows was determined by the method of enzyme-linked immunosorbent assay with commercial sets of NVO Immunotech Company (Russia) using the Uniplan AIFR-01 analyzer (Pikon Company, Russia). The time of the first clinical signs appearance and the height of bronchitis for the calves, the severity of the course and complication in the form of bronchopneumonia were taken into account. To assess the relationship between serum concentrations of hormones studied in cows and the indicators of clinical status of calves, nonparametric correlation criteria of Spearman and tau-Kendall were used. The diagnostic cut-off point of the analyzed factors was determined using ROC analysis in the IBM SPSS Statistics 20.0 program (IBM Corp., USA). It has been established that the predictor of bronchopneumonia progression of newborn calves can be the serum concentration of estradiol in their mothers. The indicator was characterized by good diagnostic value (the area under the AUC curve is 0.707), a high sensitivity of 77.8%, and a specificity of 77.3%; a critical value that cuts the risk group for bronchopneumonia progression - less than 71.2 pmol/l. To predict the early (within the first week of life) development of bronchitis of calves, high diagnostic value for estradiol concentration (AUC = 0.729) and progesterone / estradiol (AUC = 0.750) in the serum of their mothers was found: sensitivity 70,8% and 54,2%, specificity 85,7% and 85,7% respectively; critical values that cut the risk group for early development of bronchitis are less than 116.8 pmol/l and more than 571.0: 1, respectively. Thus, the determination of serum concentration of progesterone, estradiol and DHEA-C in cows 25-32 days before calving allows to predict the respiratory diseases progression among their posterity with high accuracy.

056 Gametes Biology, Embryology and Fetal Development

HSPA5 and *in vitro* embryo production: effect of addition of the protein during *in vitro* embryo culture in cattle

P.H. Santos*[1], P.K. Fontes[1], F.F. Franchi[1], A.B. Giroto[2], S.G. Nunes[3], V.B. Guilherme[3], A.C.S. Castilho[2]

[1]Universidade Estadual Paulista (UNESP), Departamento de Farmacologia, Botucatu, São Paulo, Brazil; [2]Universidade do Oeste Paulista (UNOESTE), Presidente Prudente, São Paulo, Brazil; [3]Universidade Estadual Paulista (UNESP), Departamento de Ciências Biológicas, Assis, São Paulo, Brazil.

After fertilization, the early embryo is exposed to oviductal epithelial cells and surrounded by oviductal fluid. The oviduct provides important structural, environmental and nutritional support for early embryonic development. Although oviductal fluid is not totally defined, it is known to contain HSPA5 protein. The HSPA5 is a member of the heat shock 70 class of proteins, also known as GRP78, and seems to play an important role in embryonic cell growth. Thus, the present study aimed to investigate the effect of HSPA5 during *in vitro* embryo culture on blastocyst (BL) yield. First, c*umulus*-oocytes complexes (COCs) were recovered from ovaries of slaughtered cows and *in vitro* matured in TCM199 medium plus pyruvate, amicacin, BSA (bovine serum albumin) or FBS (fetal bovine serum) in a humidified atmosphere at 5% CO_2 for 24h. After *in vitro* fertilization, presumptive zygotes derived from BSA or FBS *in vitro* maturation were denuded and cultured in a commercial culture medium in the absence (control group) or presence of HSPA5 (1 ng/mL: G1 group or 100 ng/mL: G100 group) in humidified controlled atmosphere at 5% CO_2 and O_2 until the BL stage. Therefore, experimental groups were: i) embryos derived from BSA maturation medium and cultured in control, G1 group, or G100 group (n=20 COCs/group; 7 replicates); ii) embryos derived from FBS maturation medium and cultured in control, G1 group, or G100 group (n=20 COCs/group; 7 replicates). The medium was partially replaced at day 3 and 5 with glucose addition (1µL of a 0,09mg/mL solution) on the last day feeding. The BL rates were assessed on day 7 and 8 after *in vitro* culture (IVC). Therefore, the experimental design was a 2x3 factorial, including two supplements during IVM (FBS or BSA) and three supplements during embryo culture [FBS; HSPA5 (1ng) or HSPA1 (100ng)], totalizing 6 experimental groups. Data were analyzed by ANOVA, PROC GLIMIX (SAS) and Tukey test was used to compare means. Significant differences were considered when $P < 0.05$. The BL yield from COCs matured in the BSA control group was increased compared with the FBS control group (40.05±3.35 and 25.5±4.6 respectively; $P = 0.04$), independently of HSPA addition. On day 7, embryo yield from COCs matured with BSA medium was higher in the control group (40.05±3.35) when compared with the G1 group (23±4.43; $P = 0.04$), but not different compared to the G100 group (32.62±3.8). The BL yield on day 7 plus day 8 was not affected by the presence of HSPA5 in the IVC medium: COCs matured in BSA medium (control: 43.07%; G1: 45.09% and G100: 46.6%) or COCs matured in FBS medium (control: 50.26%, G1: 54.2% and G100: 54.6%). However, the percentage of BL was higher in those derived from COCs matured in BSA and cultured with 100 ng/mL of HSPA5 protein ($P = 0.01$; 59±3), compared to those derived from COCs matured in BSF medium (46.67±2.46). Taken together these results suggest that HSPA5 may have a positive effect on blastocyst yield when COCs are matured only with BSA. Financial support: FAPESP (grant #2013/11480-3).

057 Gametes Biology, Embryology and Fetal Development

Effect of laterality of ovulation and the presence of embryo on uterine horn irrigation in llamas

M.E. Silva*[1], F. Urra[2], M.H. Ratto[2]

[1]School of Veterinary Medicine, Núcleo de Investigación en Producción Alimentaria, Universidad Católica de Temuco, Temuco, Chile; [2]Faculty of Veterinary Sciences, Universidad Austral de Chile, Valdivia, Chile.

Llamas have several unique reproductive characteristics. The establishment of pregnancies almost exclusively in the left uterine horn, regardless of laterality of ovulation, is one of them. Llama uterus presents differences in the vascular irrigation and drainage arrangements between both uterine horns (Del Campo MR, Del Campo CH, Ginther OJ. 1996. Vascular provisions for a local utero-ovarian cross-over pathway in new world camelids. *Theriogenology* 46:983-991). The presence of a prominent cross-over arterial branch extending from the right uterine artery to the left horn suggests that this is irrigated with a greater blood flow. Since an adequate endometrial blood supply is essential for a successful embryo implantation (Raine-Fenning N. 2008. Doppler assessment of uterine artery blood flow for the prediction of pregnancy after assisted reproduction treatment. Ultrasound Obstet Gynecol, 31:371-375), studies on the spatial relationship between the location of the early embryo and the degree of endometrial vascular perfusion in llamas are required. The aim of this study was to determine if intrauterine embryo location differentially induces changes in endometrial vascular perfusion between left and right uterine horns, during and after embryo migration, elongation and implantation in llamas. Adult, non-pregnant and non-lactating llamas (n = 24) were subjected to daily B-mode ultrasound scanning of their ovaries. Llamas with a growing follicle ≥8 mm in diameter in the left (n = 12) and right (n = 12) ovary were assigned to a single mating with a fertile adult male. Color-Power Doppler ultrasonography was used to determine the area of endometrial vascularization (AEV) in a cross section of the middle segment of both uterine horns. AEV was determined by off-line measurements of the average of three still images of each horn using the ImageJ software. AEV measurements were performed before mating and on days 5,10,15,20,25 and 30 post copulation in pregnant (n = 6 llamas with left or right ovulations) and non pregnant females (n = 6 llamas with left or right ovulations). Also, during the first 48 hours after mating, ovulation was confirmed by the disappearance of a follicle (≥8 mm) detected previously. Pregnancy was confirmed by the presence of the embryo proper and heart beat was used as a sign of embryo viability. AEV were analyzed by one-way ANOVA for repeated measures using the MIXED Procedure in SAS. If significant ($P \leq 0.05$) main effects or interactions were detected, Tukey´s post-hoc test for multiple comparisons was used. First observation of uterine content and embryo (exclusively in the left uterine horn) was on day 15.8 ± 3.8 and 22 ± 2.7, and 16.7± 2.6 and 27.5 ± 2.8 for pregnant llamas ovulating in the right and left ovary respectively. Although the AEV of both uterine horns was affected by time ($P < 0.05$) in pregnant llamas, it was not affected by the presence of embryo ($P = 0.35$) or laterality of ovulation ($P = 0.4$). Contrary to expected, regardless of the laterality of ovulation, in pregnant llamas the left horn did not display a greater AEV before or after embryo arrival. This trent was observed during the entire evaluation period. Proyecto Fondecyt Iniciación 11140396 (M. Silva) y Regular 1160934 (M. Ratto).

058 Gametes Biology, Embryology and Fetal Development

Treatment of bovine embryos with dickkopf-related protein 1 from the morula to blastocyst stages of development alters trophoblast elongation at day 15 of pregnancy

P. Tribulo*[1], M. Bonnet Bo[2], L.R. Carvalheria[3], J.V. Bishop[4], T.R. Hansen[4], P.J. Hansen[1],

[1]Department of Animal Sciences, D.H. Barron Reproductive and Perinatal Biology Research Program, and Genetics Institute, University of Florida, Gainesville, Florida, USA; [2]Embriovet S.L., A Coruña, Spain; [3]Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [4]Animal Reproduction and Biotechnology Laboratory. Department of Biomedical Sciences. Colorado State University, Fort Collins, CO, USA.

Dickkopf-related protein 1 (DKK1) is an endogenous inhibitor of WNT signaling produced by the endometrium which can improve competence of in-vitro produced embryos to establish and maintain pregnancy after transfer into recipients. Here, it was tested whether actions of DKK1 from days 5 to 7 of development (when the embryo is a morula and blastocyst) improved the ability of the embryo to undergo trophoblast elongation at day 15 of pregnancy. Embryos were produced in-vitro using oocytes from slaughterhouse ovaries and were exposed to 0 or 100 ng/ml recombinant human DKK1 from day 5 to 7.5 of culture. Blastocysts were transferred into synchronized recipient cows on Day 7.5 (n = 23 for control and 17 for DKK1). On Day 15, cows were slaughtered and conceptuses recovered by flushing the uterus with 50 to 200 mL Dulbecco's phosphate buffered saline (DPBS). Recovery was 48% (n =11) for control and 65% (n = 11) for DDK1 embryos. Except for two DKK1 embryos, all conceptuses were filamentous. Treatment with DKK1 increased (P = 0.007) the length of filamentous conceptuses from 63.7 mm (control) to 153.7 mm (DKK1). Concentration of interferon-τ (IFNT) in uterine flushings was measured using a sandwich ELISA with a limit of detection of 32.00 pg/mL and total IFNT was calculated using IFNT concentration and the infused volume of DPBS. DKK1 increased (P = 0.01) the amount of IFNT in uterine flushings of cows with filamentous embryos from 9.42 µg (control) to 21.84 µg (DKK1). It was concluded that DKK1 can act on the morula-to-blastocyst stage embryo to modify subsequent trophoblast elongation. Higher pregnancy rates associated with transfer of DKK1-treated embryos may be due in part to enhancements of trophoblast growth and antiluteolytic signaling through IFNT secretion. Support: USDA-NIFA AFRI Grant 2017-67015-26452 and CAPES, Brazil.

059 Gametes Biology, Embryology and Fetal Development

Proteome profile of extracellular vesicles isolated from culture media of pre-elongation stage bovine embryos

J. Valdes, E. Mellisho, J. Cabezas, B. Melo-Baez, S. Saez, L. Rodriguez-Alvarez*

Laboratory of Animal Biotechnology, Department of Animal Science, Faculty of Veterinary Science, University of Concepcion, Chillan, Bio Bio, Chile.

In bovine, elongation of the preimplantation embryo is a prerequisite for successful implantation, likewise it is critically dependent on the correct embryo-maternal communication. Pre-implantation bovine embryos as well as other cell types secrete extracellular (EVs) vesicles. This suggest that EVs play a role during early embryo-maternal for normal development and conceptus implantation. The aim of this study was to identify the proteome profile of EVs secreted by bovine pre-elongation stage embryos. Pools of 25 to 30 zygotes were cultured in vitro in 500 µL in 4-well plates using SOFaa medium until day 7 post-IVF. At day 7, the blastocysts in developmental stages 5, 6 and 7 according to morphological criteria of the IETS, were placed individually in SOFaa medium for extended culture until day 9 post IVF. At Day 9, only blastocysts in developmental stages 8 and 9 were further cultured individually in conditioned medium depleted of EVs (SOFdep) until day 12 post IVF. Embryo culture media from these embryos, was collected individually matching with the corresponding embryo and preserved individually at -80°C until further analysis. The EVs were isolated and characterized using nanoparticle tracking analysis (NTA). Then, all samples of embryo culture media were pooled in a single group and compared to SOFdep media (reference group). Proteomic profile of EVs was carried out by gel electrophoresis and protein identification performed by MALDI-ToF Mass Spectrometry. In all, culture media from 56 blastocysts with diameter in a range of 477.94±173.62 µm, at day 12 were pooled. The morphological characteristics of isolated EVs from culture medium were 127.38 ±36.63 nm of mean size and 2.97± 1.11 x 10^9 particles per mL. Twenty-eight proteins were identified, 13 were common, 3 were unique in reference group and 12 exclusive of the embryo culture media. The embryo-exclusive proteins present in EVS were: adiponectin, kininogen-1, fetuin-B, afamin, beta-2-glycoprotein 1, angiotensinogen, hemopexin, ceruloplasmin, vitamin D-binding protein, apolipoprotein A-II, olfactomedin 3 and alpha-2-antiplasmin. Using the Uniprot database the identified proteins were mapped to subcellular locations or to be secreted. Interestingly most of them, eight are involved in active process during embryo implantation. For instances, adiponectin is crucial for human embryo implantation (Duval *et al*., 2017), increase in hemopexin is related with a normal pregnancy (Bakker *et al*., 2007). In mice, fetuin-B protects against zona pellucida hardening, and is critical before fertilization as well as a later stages of embryonic development and might be related with embryo hatching (Dietzel, 2014). These results lead us to hypothesize that secreted EVs play a crucial role during embryo implantation in the bovine. We appreciate the input of Dr. Eduardo Callegari from University of South Dakota and Dr. Mauricio Hernandez from AUSTRAL-Omics. Work supported by Grant Fondecyt 1170310, Ministry of Education, Chile.

060 Gametes Biology, Embryology and Fetal Development

Prepuberal Sertoli cell proliferation differs between the bull and boar

T. Berger*, K. Miller

University of California, Davis, CA, USA.

Sertoli cells support spermatozoa during their development and the number of Sertoli cells is a major determinant of sperm production capacity in a male. The total number of Sertoli cells is normally determined by prepuberal proliferation. In recent decades, two waves of postnatal, prepuberal proliferation have been described in pigs and primates but not in rodents. The usual interpretation has been that two postnatal waves of proliferation are typical but waves occur concurrently or only one exists in early maturing rodents. Defining the timing of proliferative waves will assist in manipulations to increase Sertoli cell numbers and adult sperm production capacity. For example, reducing endogenous testicular estradiol stimulates the first postnatal wave of Sertoli cell proliferation in the boar but does not affect the second wave. In our first study, groups of Angus-Hereford crossbred bull calves were castrated at 1, 2, 3, 4, 5, and 6 mo of age (n = 8 per age). Testes were weighed and equatorial segments fixed. Sertoli cell density was determined following immunohistochemical labeling of Sertoli cells with GATA-4 antibody in 30- micron thick sections. The number of Sertoli cells per testis increased linearly from 1 mo to 5 mo of age ($r = 0.77$; $P < 0.001$). Sertoli cell numbers appeared to have plateaued at 5 mo of age and luminal development also was present. Hence, only one postnatal wave of Sertoli cell proliferation was detectable in the bull. In the second study, Jersey bull caves were treated twice weekly with an aromatase inhibitor, letrozole, beginning at 2 weeks of age, with the control animals receiving the canola oil vehicle. At 6 mo of age, testes were retrieved, weighed, tissue fixed and Sertoli cell density determined. Testicular tissue was analyzed for estradiol and testosterone and serum was analyzed for FSH and LH. Testicular estradiol was reduced ($P < 0.05$) in letrozole-treated bulls, as anticipated. Consistent with results in the boar, no effects on testosterone or circulating FSH or LH were observed. In contrast to the boar, reducing testicular estradiol did not alter Sertoli cell numbers. This discrepancy between responses in the boar and the bull might be due to the relatively low level of estradiol present in the testis in control animals (approximately 1% of that in boars) such that estradiol was exerting minimal inhibitory effects in the control bulls. The absence of a clearly distinguishable first postnatal wave of Sertoli cell proliferation in bulls is a second possibility for the discrepancy as only the first postnatal wave of Sertoli cell proliferation responds to reduced testicular estradiol in the boar. A larger proportion of the interval between sexual differentiation of the gonad and puberty is encompassed by gestation in the bull than in the boar; hence Sertoli cell proliferation corresponding to the first postnatal wave in the boar may be occurring prenatally in the bull, a possibility that awaits further research. (Supported by UC Davis FAPESP and AJCC).

061 Gametes Biology, Embryology and Fetal Development

Comprehensive evaluation of metabolic behavior in preimplantation embryo

C.B. Lima[2,1], E.C. Santos[1], J. Ispada[2,1], P.K. Fontes[3], M.F.G. Nogueira[3], C.M.D. Santos[1], M.P. Milazzotto*[1]

[1]UFABC - Federal University of ABC, Santo Andre, SP, Brazil; [2]USP - University of Sao Paulo, Sao Paulo, SP, Brazil; [3]UNESP - Sao Paulo State University, Botucatu, SP, Brazil.

The study of key metabolic processes of the early embryo has been driven by the desire to define requirements for embryo growth and viability. This study was designed to monitor the metabolic response of embryos with distinct kinetics when submitted to a combination of common environmental stressors in the culture system. Using a factorial experimental design (2x2x3), Fast (4 or more cells at 22 hours post insemination) and Slow (2-3 cells) embryos were produced *in vitro* by standard protocols and cultured in 20% or 5% O_2, and also in different glucose concentrations (0, 2 or 5mM), resulting in 12 study groups. Blastocysts were evaluated for 92 characters including 85 genes [control of gene expression (14 genes), lipid metabolism (16), energy metabolism (15), cell death (5), response to oxidative stress (17), other cellular functions (18)]; 7 biochemical measures: consumption of glucose, glutamate and pyruvate, production of lactate and ATP, generation of reactive oxygen species (ROS), mitochondrial activity, and lipid content by mass spectrometry. Data were normalized and gathered in a matrix that was analyzed under parsimony, with 500 replicates and Tree-Bissection Reconection as the swapping algorithm (*TNT software*). All characters were additive and equally weighted. This analysis resulted in a single optimal tree, fully resolved, which provided a panoramic view of the system. Interestingly, all groups arranged in only 2 main clusters according to high or lower oxygen tension, suggesting that this category stands out in terms of developmental kinetics and glucose, except for the groups cultured in a hyperglycemic environment (5mM) that formed a separate cluster. Further investigation of these 2 clusters (correlation attribute evaluation – *Weka software*) was able to highlight characters that most contributed to differentiate groups cultured in 20% vs. 5% O_2. A total of 33 genes [control of gene expression (8); lipid metabolism (10); cell death (2); response to oxidative stress (10)] were found to be related to such differences. In general, embryos cultured at 20% of O_2 were oriented to greater glycolytic activity, since the transcripts for glucose transporters and PFK were increased. Especially in the 2mM glucose groups, increased consumption of pyruvate, mitochondrial activity and production of ROS, indicated that apart from glycolysis, these embryos were also performing more oxidative phosphorylation than those at 5% O_2. However, this greater "commitment" to producing energy was not reflected in the amount of ATP. Moreover, at 20% O_2, the higher transcription of genes related to lipid synthesis, elongation and desaturation was accompanied by augmented lipid content, which is usually an indicator of lower quality. In contrast, at 5% O_2 the presence of palmitoyl carnitine indicated that these embryos were resorting to ß-oxidation, a highly efficient mode of energy production for the cell. Considering the plasticity of embryonic responses, our results help unravel the complexity of culture system and indicate that oxygen tension is an important factor for the maintenance of proper metabolic status. Sao Paulo Research Foundation #2015/03381-0.

062 Gametes Biology, Embryology and Fetal Development

Differential transcript profiles in cumulus-oocyte complexes originating from pre-ovulatory follicles of varied physiological maturity in beef cows

S.E. Dickinson*[1], T.W. Geary[2], J.M. Monnig[1], J.A. Green[1], K.G. Pohler[3], G.A. Bridges[4], S. Behura[1], M.F. Smith[1]

[1]University of Missouri, Columbia, MO, USA; [2]USDA-ARS, Fort Keogh Livestock and Range Research Lab, Miles City, MT, USA; [3]University of Tennessee, Knoxville, TN, USA; [4]University of Minnesota, St. Paul, MN, USA.

Dominant follicle diameter at exogenous gonadotropin releasing hormone (GnRH) induced ovulation, but not at spontaneous ovulation, influenced pregnancy rate and late embryonic mortality in postpartum beef cows. Furthermore, induced ovulation of a larger dominant follicle increased fertilization rate and the probability of recovering a transferable embryo on day 7 post insemination. Therefore, we hypothesized that the physiological status of a pre-ovulatory follicle at GnRH-induced ovulation has a direct effect on oocyte competence and subsequent embryo development. The objective of this study was to determine if the transcriptome of oocytes and associated cumulus cells (CC) differed between GnRH-induced small follicles (<11.7mm), GnRH-induced large follicles (>12.5 mm), or endogenous gonadotropin surge, spontaneous follicles (11.6-13.9 mm). Ovulation was synchronized in postpartum beef cows (n=250), and dominant follicles were trans-vaginally aspirated ~20 hours after GnRH-induced ovulation (small; large) or the onset of estrus (spontaneous). Oocytes and CC were individually snap-frozen from each cumulus-oocyte complex (COC). For library preparation, RNA was extracted from pools of 4 oocytes or CC from 4 COCs (n= 6 pools for small and large follicles; n=5 pools for spontaneous follicles). An average of 21 million uniquely aligned, single end reads per sample were generated and differential expression analysis between sample groups was performed by fitting the expression data to a general linear model using edgeR robust (significance determined at FDR<0.10). Comparisons of the oocyte transcriptome revealed relatively few differentially expressed genes (DEG; 11, 15, and 9), whereas 884, 1609, 1491 DEG were revealed between CC of small vs. large, small vs. spontaneous, and large vs. spontaneous follicles, respectively. Most noteworthy, when CC from small and large follicles were compared, 430 transcripts were more abundant in CC from small follicles and 454 were more abundant in CC from large follicles. Interrogation of these transcripts within KEGG pathways revealed significant enrichment of 'Glycolysis/Gluconeogenesis' (FDR<$2.2x10^{-5}$) in CC from large compared to small follicles. Oocytes have a poor capacity for metabolizing glucose and rely on CC to supply pyruvate for energy production necessary for maturation. Therefore, we conclude that an inefficient or immature glycolytic pathway in cumulus cells from small follicles, alongside additional DEGs and associated pathways, contributes to the decreased competency of oocytes from small follicles in comparison to large or spontaneous follicles. AFRI Grant no. 2013-67015-21076 from the USDA National Institute of Food and Agriculture.