A206 Embryology, Developmental Biology and Physiology of Reproduction

Transcription factors activated by 17B-estradiol signaling in corpus luteum of non-pregnant bitches

A.P.M.M. Cardoso[1], R. dos Santos Silva[1], I.C. Giometti[2], P.C. Papa[1]

[1]USP, Sao Paulo; [2]UNOESTE, Presidente Prudente.

Keywords: estradiol, corpus luteum, RNAseq.

The canine corpus luteum (CL) is responsible for E2 synthesis during diestrus, which acts in an autocrine or/and paracrine way in this gland. The acting mechanism depends on expression ratio between alfa (ESR1) and beta receptor (ESR2). The binding to ESR1 has a proliferative and to ESR2 an antiproliferative effect. The aim of this study was to better understand the signaling mediated by ESR1 and ESR2 in the formation and regression of CL. The CLs were obtained from non-pregnant bitches (n = 30) on days 10, 20, 30, 40, 50 and 60 (n = 5/group) post-ovulation (p.o.). On the ovariossalpingohisterectomy day, blood was collected to measure P4 and E2 concentration. Eighteen CLs (n = 3/group) were submitted to RNA sequencing (RNAseq). The differential expressed (DE) genes identified by RNAseq were submitted to oPOSSUM3 (http://opossum.cisreg.ca) software for the detection of over-represented transcription factor binding sites (TBFS). Twelve DE genes were selected for validation of the RNA seq results through qPCR, and GAPDH was used as reference gene. From those genes, four have showed TFBS with ESR2 (lymphoid enhancer-binding factor 1 - LEF-1; pregnancy-associated plasma protein A - PAPPA; ; N-myc downstream-regulated gene 2 - NDGR2; ATPase Na+/K+ - ATP1A1) and others participate in the control of cell proliferation (beta-catenin - CTNNB1; ciclin D1 - CCND1; Axin 2; insulin binding proteins - IGFBP 3, 4 e 5; platelet derived growth factor subunit B - PDGFB). The relative gene expression was calculated by Pfaffl method (Nucleic Acids Research, 29: 2004-2007, 2001). The statistical analysis was carried out by ANOVA one-way following Bonferroni test ($P < 0.05$). The correlation between parameters was checked by Pearson correlation ($P < 0.05$). There was a positive correlation, and values varied from 0,375 (CCND1) to a maximum of 0.9984 (PAPPA), with average value of 0,735. No significant difference was observed for CTNNB1, LEF-1, CCND1, Axin2, IGFBP5, NGDR2, ATP1A1 and PDGFB gene expression. The PAPPA, IGFBP3 and IGFBP4 gene expression decreased significantly throughout diestrus, presenting the highest value on the 10 day ($P < 0.05$). The CAV-1 gene expression showed no variation in the CL development and maintenance phases (10 and 40 days p.o), but decreased significantly during its regression, showing the lowest value on day 60p.o. The P4 highest value was found on day 20 p.o. compared to other days and day 30 showed higher values than days 50 and 60 p.o. ($P < 0.05$), E2 showed increasing values until day 50 p.o.(beginning of CL structural regression) and then ($P < 0.05$) decresased. We conclude that RNAseq is an effective methodology for DE gene detection, especially for genes with low expression and that E2, possibily, contributes to regulation of CL function through interactive mechanisms involving IGF system and caveolin-1.

A207 Embryology, Developmental Biology and Physiology of Reproduction

Differential expression analysis of components of the insulin signaling cascade in the canine corpus luteum after RNA sequencing

R. dos Santos Silva[1], A.P.M.M. Cardoso[1], S. Unniappan[2], I.C. Giometti[3], P.C. Papa[1]

[1]University of São Paulo/Depart. of Surgery, School of Veterinary Medicine and Animal Science, São Paulo; [2]University of Saskatchewan, Saskatoon; [3]UNOESTE, Presidente Prudente.

Keywords: corpus luteum, insulin, RNAseq.

The present study aims to characterize the expression of genes regulated by insulin in the canine corpus luteum (CL) along the diestrus. Hypothesis is that insulin plays a role in the regulation of steroidogenesis and the functions of lutein cells, consequently. Non-pregnant bitches were submitted to ovariosalpingohisterectomy every 10 days between 10 and 60 (n = 4 / group) after ovulation (po). CL collected were used for RNA sequencing (RNA-seq) and validated by real-time PCR, and protein analysis by Western blotting and immunofluorescence. Bioinformatics analysis detected the presence of 34.408 genes in the samples, of which 29.011 genes showed some level of gene expression in diestrus. The temporal gene expression revealed the presence of 5.116 genes differentially expressed at least one comparison between groups and 1.106 genes were not noted in the canine genome. Seven genes directly linked to insulin signaling were selected: the IRS1 (insulin substrate 1 receptor), involved with the onset of insulin signaling, the PI3KR3 (phosphoinositide-3-kinase, regulatory subunit 3 (gamma) and PI3KCG (phosphoinositide-3-kinase , catalytic, gamma polypeptide) that are PI3K isoforms involved in phosphorylation of AKT, the MAPK9 (mitogen-activated protein kinase 9), MAPK13 (mitogen-activated protein kinase 13), and MAPK14 (mitogen-activated protein kinase 14) involved in the cell proliferation and SOCS1 (suppressor of cytokine signaling 1), known as a positive regulator of insulin signaling initiation. The expression of IRS1, MAPK13 and MAP14 was higher in the second half of diestrus, concomitant with decreased expression of steroidogenic enzymes HSD3B and CYP7B1, suggesting a negative regulatory performed by these genes. Rather, PI3KCG, PI3KR3, MAPK9 and SOCS1 expression have maximum values at the beginning of estrus, similar to the CYP19A1 expression, suggesting a positive control. The results corroborate with studies in which the different components involved in the insulin signaling establish a direct interaction with the expression of steroidogenic enzymes, from low molecular weight mediators such as inositolphosphoglycan. After the association of insulin with its receptor, the mediators of this class are generated, internalized and alter the activities of some steroidogenic enzymes and cellular metabolism, in addition to acting in the cleavage of cholesterol side chain resulting in increased production of hormones. These data allow a better understanding of the regulation of luteal physiology in general and more specifically on the regulation of steroidogenesis through insulin signaling.

A208 Embryology, Developmental Biology and Physiology of Reproduction

Effects of intrammamary LPS infusions on inflammation and reproductive parameters of Holstein dairy cows

C.C. Campos[1], R.M. Santos[1], R.L.A. Cerri[2]

[1]Universidade Federal de Uberlândia, Uberlandia; [2]University of British Columbia, Vancouver.

Keywords: embryo development, LPS, mastitis.

The objective was to evaluate the effects of LPS induced mastitis on systemic inflammatory response and early embryo development in lactating Holstein cows. Cows at 35 ± 7 DIM (n = 20) were submitted to a modified Double-Ovsynch program (Souza, Theriogenology, v.70, p.208-2015, 2008) (10 days interval between protocols with CIDR and two $PGF_{2\alpha}$ injections in the second protocol) and timed AI (D0). Cows were randomly assigned to two treatments: 1) LPS group - cows received an intrammamary infusions (D5 and D10) of 25 μg of LPS (strain 0111:B4, Sigma Aldrich®, St. Louis, MO) diluted in 10 ml of sterile saline at morning milkings; and 2) Control group - cows received infusion with saline. Blood samples were taken at different time intervals during the study to determine plasmatic concentrations of haptoglobin (Hp), tumoral necrosis factor alpha (TNF-α) and progesterone (P_4). Milk samples were collected for somatic cell count (SCC) at infusion milking and the next three subsequent milkings (0, 10, 24 and 34 hours), as well as milk production was evaluated. Body temperature of the cows was recorded using a rumen-reticular bolus logger and summarized for every hour during the period. On day 15 after AI (D15), uterine flushing for embryo recover and interferon-tau (IFN-τ) measurement. Data were analyzed using the MIXED procedure of SAS ($P < 0.05$). Hp was greater in LPS compared with Control group (0.80 ± 0.06 vs 0.45 ± 0.07; $P < 0.01$), but TNF-α concentration was similar ($P = 0.72$) between treatments. Milk production from d0 to d15 was greater for Control cows (37.5 ± 1.5 vs 33.5 ± 1.3 kg/d; $P < 0.01$), whereas SCC was higher in LPS treated cows for about 34 h after each infusion ($P < 0.01$). Likewise, reticular temperature of LPS cows was elevated for 12 h ($P < 0.01$) after each infusion. Progesterone did not differ among treatments at all time collections ($P = 0.72$). However, pregnant cows had greater concentrations of progesterone on d 6, 9 and 10 post-AI ($P < 0.01$). The recovery rate was 55% and the length of recovered embryos (3.6 ± 0.9 vs 2.4 ± 0.7 cm; $P = 0.56$) and IFN-τ concentration in the luminal uterine flushing ($P = 0.44$) were similar between treatments. In summary, the intrammamary infusion of LPS was able to trigger a systemic inflammatory response during post-AI period, but unable affect conceptus recovery and length, and intraluminal uterine IFN-t concentration.

A209 Embryology, Developmental Biology and Physiology of Reproduction

Embryo resilience to sublethal stress induced by high gaseous pressure

B.S. Becker[1], F.J.F. Collares[1], D.D. Mendonça[2], M. Bertolini[1], J.L.R. Rodrigues[1]

[1]UFRGS, Porto Alegre; [2]PUCRS, Porto Alegre.

Keywords: high gaseous pressure, sublethal stress, murine embryo.

Many studies have reported high pressure as a stressor that can be used to induce sublethal shock response on embryos, providing cell protection to a subsequent stress, such as cryopreservation (Pribenszky *et al*., Biol Reprod (83) 690-697, 2010; Rodrigues *et al*., Reprod Fertil Dev (25) 282-283, 2012). The aim of this experiment was to investigate the use of high gaseous pressure (HGP) as an alternative to high hydrostatic pressure in order to induce murine blastocyst resilience. A total of 47 (69.1 %) out of 68 superovulated *Mus musculus domesticus* females, produced 518 blastocysts that were segregated into three experimental groups: (a) 20.7 MPa for 2 h (P20.T2); (b) 20.7 MPa for 4 h (P20.T4); and (c) 34.5 MPa for 2 h (P34.T2). All groups were paired with control blastocysts non-exposed to HGP. Embryos were exposed to HGP and after submitted to *in vitro* culture in mKSOM media + 0.4% BSA for 72 h at 37ºC, 5% CO2, 5% O2, 90% N2 and satured humidity. Hatching rates were compared between control and experimental groups by Chi square test ($P < 0.05$). No difference was observed in hatching rates between experimental and control groups: (a) P20.T2 84.5% (60/71) vs. control 86.2% (81/94); (b) P20.T4 93.5% (131/140) vs. control 88.2% (75/85); and (c) P34.T2 74/78 (94.9%) vs. control 92.0% (46/50). Therefore, we concluded that HGP can be used as sublethal stressor without compromising *in vitro* embryo viability. Gene expression analyses are underway to identify embryo molecular modifications or markers induced by HGP.

A210 Embryology, Developmental Biology and Physiology of Reproduction

Recovery of reproductive activity and fertility of Saanen goats affected by hydrometra after cloprostenol treatment and estrus induction during the non-breeding season (preliminary data)

J.F. Fonseca[1], A.L.R.S. Maia[2], F.Z. Brandao[2], M.F.A. Balaro[2], G.B. Santos[2], J.M.G. Souza Fabjan[2], R.I.T.P. Batista[2], O. Facó[1]

[1]EMBRAPA Caprinos e Ovinos, Coronel Pacheco; [2]Universidade Federal Fluminense, Niterói.

Keywords: reproductive efficiency, hydrometra, goat.

The hydrometra is one of the most important causes of subfertility or infertility in dairy goats. The pseudo-pregnant goats are normally recognized only after five months of expected gestation because partum does not occur. On the other hand, ultrasonography (US) can easily diagnose hydrometra both sooner or latter. Sometimes hydrometra affected goats also show concomitant follicular cysts, which will only be seen after first cloprostenol administration and uterine drainage. This study evaluated uterine drainage and fertility in hydrometra affected goats after use of d-cloprostenol treatment associated or not to GnRH followed by estrous induction. The study was performed during the non-breeding season of 2015 in Minas Gerais, Brazil. A total of 10 from 18 Saanen goats (55.5%) was diagnosed with hydrometra, aged 18 to 102 month-old and showing body condition score (BCS) from 2.75 to 4.25 (scale 1 to 5). These goats received three doses of 37.5 µg d-cloprostenol (Prolise®, Tecnopec LTDA, São Paulo, Brazil) laterovulvarly 10 days apart (D0, D10 and D20). At D5, goats were randomly assigned into two groups to receive 1 mL (25 µg) i.m. GnRH (Gestran Plus®, Tecnopec LTDA, São Paulo, Brazil) or 1 mL 0.9% saline i.m., respectively. At D25, goats received 60 mg MAP sponges for six days (Progespon ®, Shering-Plough Animal Health, São Paulo, Brazil) plus 37.5 µg d-cloprostenol laterovulvar and 200 IU eCG (Novormon 5000 ®, Shering-Plough Animal health, São Paulo, Brazil) i.m., 24 hours before sponge removal. Transrectal US (Mindray® 330DP, Shenzhen, China) was performed 60 days after estrus induction to check pregnancy. Data are presented in a descriptive form. All goats displayed estrus after estrus induction. US at 60 days after breeding revealed 50% of pregnancy rate (5/10), being 80% to GnRH (4/5) and 20% to Control (2/5) treated goats. In goats that were not pregnant, it was noted one with fetal loss (Control) and one that showed hydrometra again (GnRH). The remaining goats showed complete uterine drainage (Grade 0 uterus). The use of three doses of d-cloprostenol 10 days apart followed by estrus induction was efficient for draining the contents of the uterus resulting in a relative good pregnancy rate.

Financial support: CNPq – Project 479826/2013-7 and Fapemig -Project CVZ-PPM 00042-14.

A211 Embryology, Developmental Biology and Physiology of Reproduction

A simple technique for quantifying apoptosis of *in vitro* matured oocytes in cows

A. Velasquez, E. Mellisho-Salas, J. Cabezas, M.J. Nuñez, L. Rodriguez-Alvarez

Laboratory Of Animal Biotechnology, Department Of Animal Science, Faculty of Veterinary Science, Uni, Chillán, Region Bio Bio, Chile.

Keywords: apoptosis, oocytes, cows.

The apoptosis is characterized by cytoplasmic and nuclear fragmentation, chromatin condensation and DNA fragmentation. Currently available methods for quantifying apoptosis have various limitations: (a) different staining methods involving multiple steps, (b) difficulty for quantifying cell fluorescence and (c) non-specific detection. The objective of this study was to quantify apoptosis of in vitro matured of dairy and beef cattle, using acridine orange/propidium iodide (AO/PI) and counting of total fluorescence in a cell using image J software. Ovaries of dairy and beef cows were collected at an abattoir and transported within 2 hours to the laboratory. Cumulus–oocyte–complexes (COCs) were recovered by follicle aspiration with a 20G needle and syringe. The COCs recovered (quality 1 and 2) were matured individually for 23 h at 39˚C under 5% CO_2. In order to quantify the cumulus expansion, the cumulus area was measured in individual COCs before and after in vitro maturation. The matured oocytes were incubated at 37 ˚C in 10 ul of PBS supplemented with dual fluorescent staining solution (AO/PI, Logos Biosystems) for 5 minutes before visualization. The morphology of apoptotic cells was examined and counted within 10 min using inverted fluorescent microscope (EVOS TL, Thermo Fisher Scientific Inc) and processed with image J software v 1.43u (https://imagej.nih.gov/ij). ImageJ is available in the public domain (i.e., free). The overlay images were captured in TIFF file format with inverted fluorescent microscope with GFP and RFP channel. Images were converted to greyscale of 8 bits and different channels were selected to measure fluorescence. Cell of interest were selected using any of the drawing/selection tools and analyzed by measuring area, mean gray value and integrated density. The region next to the cell that has no fluorescence was considered as background. Comparison analyses between groups were performed by analysis of variance (ANOVA) using InfoStat program (Buenos Aires, Argentina). Differences were considered significant at $P < 0.05$. The area of COCs from dairy (71 150 μm^2) and beef (110 745 μm^2) cows, before in vitro maturation showed no significant differences (P = 0,074). Apoptosis index of cumulus cells of oocytes matured in vitro in dairy (26,3%) and beef (30,6%) cows showed no statistical difference (P = 0,65). Nevertheless, in vitro matured COCS for 23 h of beef cows (781 382 μm^2) had greater expansion (7.05 versus 4.7 times) that COCs of dairy cows (337 832 μm^2) (P = 0,045). In conclusion, the protocol using acridine orange/propidium iodide (AO/PI), is the simple and quick method for measuring apoptosis in cumulus cell of oocyte. The program image J complements the work of staining fluorescence and makes possible the measurement of the fluorescence of a cell in an objective manner.

A212 Embryology, Developmental Biology and Physiology of Reproduction

Pregnancy in a heifer after bilateral ovariectomy

C.B. da Silva, K.C. Silva-Santos, G.M.G. Santos, A.C.O. Dearo, M.M. Seneda

Universidade Estadual de Londrina, Curitiba.

Keywords: ovariectomy, gestation, Nelore.

A 24-month-old Nelore heifer became naturally pregnant after undergoing a bilateral ovariectomy. This unexpected event occurred after an experiment that was conducted to estimate the preantral follicle populations in calves' ovaries. Fourteen female calves aged 8 months were bilaterally ovariectomized by laparotomy. The procedures were performed in a surgical suite under sedation and a paravertebral block. Both ovaries of each calf were carefully collected, identified, measured, weighted, photographed and processed for histology and follicle counting. Through all steps the ovaries integrity was assessed for visual and microscopy criteria. After those procedures, the calves were housed at an experimental farm with some bulls. Sixteen months later, the heifers were used in a study using intravaginal progesterone devices and evaluation of hormonal levels. Surprisingly, one heifer showed 6.11 ng/mL of serum progesterone (radioimmunoassay, Coat-a-Count) before the insertion of the intravaginal device. A transrectal ultrasound examination revealed an "ovary-like" structure, and a pregnancy was confirmed. The pregnancy progressed uneventfully, and the heifer delivered a healthy male calf. After weaning, the heifer was slaughtered; the reproductive tract was collected, photographed and evaluated. The presumptive ovary was histologically assessed. The parenchyma presented an unusual pattern, with predominance of connective tissue, with a low number of follicles at different stages, along with a corpus luteum. We describe this intriguing event to discuss possible explanations for this fact.

A213 Embryology, Developmental Biology and Physiology of Reproduction

Doppler ultrasound in the diagnosis of early pregnancy in sheep

G.M. Bragança[1], M.F.A. Balaro[1], J.F. Fonseca[2], P.H.N. Pinto[1], R.M. Rosa[1], L.S. Ribeiro[1], M.S. Almeida[1], J.M.G. Souza Fabjan[1], A.R. Garcia[3], F.Z. Brandao[1]

[1]Universidade Federal Fluminense, Rio de Janeiro; [2]EMBRAPA Caprinos e Ovinos, Sobral; [3]EMBRAPA Pecuária Sudeste, São Carlos.

Keywords: ultrasonography, pregnancy, luteal vasculatization.

The diagnosis of early pregnancy increases reproductive efficiency because non-pregnant females can be subjected for a promptly mating, concentrating births and improving the farm management. The aim of this study was to determine the effectiveness in the diagnosis of early pregnancy on the 12nd and 17th day after sheep mating. The study was carried out in a meat sheep flock located at Cachoeiras de Macacu city, Rio de Janeiro, Brazil. A total of 58 crossbred (Dorper x Santa Inês) ewes had their estrus synchronized by two doses of 0.12 mg cloprostenol (Estron®, Agener Union Animal Health, São Paulo, Brazil) seven days apart. Estrus behavior was detected after 36 hours of the second dose and ewes were mated by healthy rams. At the 12th (pre-luteolysis), 17th (post-luteolysis) and 30th day after mating, ultrasound exams were performed aided by Color Doppler ultrasound system (Sonoscape S6®, Sonoscape, Yizhe Building, Yuquan Road, Shenzhen, China), using a 7.5 MHz linear transducer (transrectal) to assess the quantity and quality of corpora lutea (CL). The quality of CL was evaluated subjectively by the vascularization degree: 1 (0-25%), 2 (25-50%), 3 (50-75) and 4 (75-100%) in accordance with previous study (Figueira, L. Reproduction in Domestic Animals, v.50, p. 643-50, 2015). At the 30th day, pregnancy was confirmed by the uterine fluid and embryo. To calculate the sensitivity, specificity and accuracy of the pregnancy diagnosis at the 12th and 17th day (predictive diagnosis) compared to the 30th day (confirmatory diagnosis), it was considered as "not pregnant" female, ewes with a vascularization degree 1. At 17th day, in addition with the luteal vascularization, the presence or absence of uterine fluid was also observed on the categorization of pregnancy. The degree of luteal vascularization on pregnancy (positive x negative) was evaluated by Wilcoxon test (signed-rank test) at 5% significance level. An effect of the vascularization luteal degree was found on the diagnosis of gestation ($P < 0.05$). At the 12th day and 17 th day, respectively: 84.1% and 70.5% of sensitivity, 42.7% and 85.7% of specificity and 74.1% and 74.2% of accuracy were obtained in the adoption of the degree of luteal vascularity for pregnancy prediction. When associating these data of vascularization degree with the presence or absence of uterine fluid for pregnancy prediction, 100% of sensitivity, specificity and accuracy were achieved. In conclusion, the pregnancy diagnosis performed on the 12th day post-mating may correctly predict approximately 85% of the pregnant ewes. However, on the 17th day, the association of the degree of luteal vascularization and the presence of intrauterine fluid contributed to reach 100% of accuracy in the pregnancy diagnosis. Therefore, the Doppler ultrasound evaluation at the 17th day post-mating in crossbred sheep is effective in the detection of the early pregnancy.

Financial Support: CNPq, Faperj and Embrapa (Project 02.13.06.026.00.00).

A214 Embryology, Developmental Biology and Physiology of Reproduction

Early blastulation of bovine embryos, improves post hatching *in vitro* development

E. Mellisho-Salas, A. Velasquez, L. Rodriguez-Alvarez

Laboratório de Biotecnologia Animal, Departamento de Ciência Animal, Faculdade de Veterinária da Uni, Chillán, Region Bio Bio, Chile.

Keywords: blastulation, embryo, bovine.

Blastocyst formation is an essential event in preimplantation development. The post hatching development system is very useful tool for evaluation of bovine embryo viability. The objective of this study was to evaluate blastulation time as predictor of bovine embryo competence. Cattle ovaries were collected at an abattoir and transported within 2 hours to the laboratory. Cumulus–oocyte–complexes (COCs) were recovered by follicle aspiration with a 20G needle and syringe. The recovered COCs (quality 1 and 2) were matured in four-well dishes (30 COCs per 500µL well) for 22–23 h. Oocytes were fertilized using frozen–thawed semen that was used before in other in vitro fertilization procedures with good blastocyst production (control bull). Presumptive zygotes were in vitro cultured (IVC) in groups in four-well plates (30 zygotes per 500µL well) using SOFaa culture medium at 39˚C under 5% CO_2, 5% O_2 and 90% N_2 until morula stage (day-5 post IVF). The morula were cultured individually in 96 well at 39˚C under gas mixture (CO_2, O_2 and N_2) until blastulation time (day-6,5 and 7,5) for individual monitoring and analysis of post hatching development (day 11). Firstly, it was determined the hatching rate from day-6.5 and day-7.5 derived blastocysts. The post hatching development was assessed considering two variables; size (>250 um) and total cell count (>350 blastomeres). Comparison analyses between groups were performed by test of two independent proportions while correlation analyses were performed by Pearson test using InfoStat program (Buenos Aires, Argentina). Differences were considered significant at $P < 0.05$. At day 5 post IVF, the morula rate was 35.7% (278/778). Early blastulation rate (day-6.5) was 40.3% (112/278) while late blastulation rate (day 7.5) was 20.5% (57/278), showing a significant difference ($P < 0,0001$). Embryos derived from early blastulation (day 6.5) have a higher probability (39.3%: 44/112) of post hatching development (day 11) versus late blastulation (day-7,5; 10.5% (6/57); $P = 0.0001$). Also, an early blastulation show a middling correlation ($r = -0.30$; $P = 0.0001$) with post hatching development (day 11). The diameter of the blastocyst (158,3 ± 17,9 µm; early blastulation and 166,8 ± 24,5µm; late blastulation) had a low correlation with post hatching development ($r=0,16$; $P = 0,04$). At Day-11, embryos derived from early blastocysts have a higher dimeter and total cell number (425µm; 654 cells) than those derived from late blastocysts (367µm; 595 cells) ($P < 0.05$ for both parameters). In conclusion, blastulation time affects the future development of the bovine embryo and the early blastulation could be a simple tool to improve embryo selection.

A215 Embryology, Developmental Biology and Physiology of Reproduction

Characterization and comparative profiling of small RNAs in oviductal extracellular vesicles along the estrous cycle

C.A. Alminana Brines

INRA, Nouzilly.

Keywords: extracellular vesicles, oviduct, gamete/embryo-maternal communication.

Extracellular vesicles (EVs), exosomes and microvesicles, have been suggested as new regulatory vehicles that can mediate gamete/embryo-oviduct interactions by transferring selected RNAs and proteins, contained in their molecular cargo, among them. Since the oviductal epithelium is regulated by estrogen and progesterone along the estrous cycle, affecting RNAs expression and protein secretion into the oviductal fluid, we hypothesize that they may have also an impact on EVs content and secretion into the oviduct. Thus, the objective of our study was to decipher the oviductal EVs small RNA signature along the oestrous cycle and their possible action on gametes and embryos. Pairs of oviducts with their attached ovaries were collected from cyclic (corpus luteum (CL) present) cow reproductive tracts at a local abattoir. The stage of the oestrous cycle was assessed by CL morphology and follicle populations: Stage 1: recently ovulated follicle; Stage 2: early CL development; Stage III: yellow or orange CL and Stage 4: regressing CL and a large preovulatory follicle present. Subsequently, oviducts were flushed to collect their fluid. EVs were isolated by ultracentrifugation. Total RNA was isolated from EVs samples (n = 20 samples, 4 stages, 5 replicates) using miRNeasy Mini kit (QIAGEN). Small RNA libraries were prepared by using NEXTflexTM Small RNA-Seq Kit (Bioo Scientific) and subsequently sequenced on an Illumina HiSeq 2500. The obtained sequence data was processed using an established analysis pipeline integrated in a local Galaxy installation. About 250 different small RNA and ribosomal RNA genes were identified. Among them, we found 150 microRNAs (miRNA), 22 transfer RNAs (tRNA) and 45 small nucleolar RNAs (SNORD, SNORA). In addition, small RNAs involved in protein translation (7SL), regulation of transcription (7SK), and DNA replication (Y RNA) were identified. Comparative profiling of small RNAs in oviductal EVs revealed differential expression profiles along the bovine oestrous cycle (normalization and statistical analysis in Bioconductor R package EdgeR). Twelve miRNAs were found as differentially expressed across stages (ANOVA; FDR 5%). Among them, miR-10b involved in activation of cell proliferation and miR24-2 in regulation of genes related to survival pathways, were identified and associated to early embryo development. MiR-449a, associated to male and female infertility was also identified. Our study represents the first screening for small RNA in oviductal EVs. The characterization of oviduct-derived EVs under different hormonal regulation will extend our understanding of EVs regulatory processes in gamete/embryo-maternal interactions and their possible action on the regulation of gene expression in the developing embryo.

Supported by EU-AgreenSkills267196, INRA-PHASE seeding funds, EU-FECUND312097 and COST Action-EPICONCEPT FA1201.

A216 Embryology, Developmental Biology and Physiology of Reproduction

Regulation of oxidative stress response by miRNA in bovine *in vitro*-produced blastocysts

M.M. Bomfim, J.C. Silveira, F.V. Meirelles, F. Perecin

Universidade de São Paulo, Pirassununga.

Keywords: MicroRNA, ROS, embryo development.

In vitro culture (IVC) of bovine embryos exposes the conceptus to harmful conditions during embryonic development. The high tension of oxygen in IVC leads to worsening of oxidative stress by increasing reactive oxygen species (ROS) amounts. Activation of cell stress pathway can be modulated by post-transcriptional regulators, such as c. Non-coding RNAs of 18-26 nucleotides in length, known as miRNAS, act as post-transcriptional regulation of gene expression, and potentially modulate cellular responses to oxidative stress. The comprehension of molecular mechanisms driving cellular responses to oxidative stress through miRNAs might help to clarify the effects of IVC in cattle. Our hypothesis is that miRNAs are involved in regulating genes associated to oxidative stress responsive pathways. This study aim to determined miRNAs involved in pathways of oxidative stress response in two in vitro culture environment, high (20%) and low (5%) oxygen tension. Bovine blastocysts (pools of 10) produced by IVF and culture in different O2 tensions had the total RNA extracted using miRNeasy mini kit. Reverse transcription was performed using miScriptII RT allowing for mature and precursor miRNAs. Samples were analyzed for 348 miRNAs and 3 endogenous (RNU43 snoRNA, Hm/MS/Rt T1 snRNA and bta-miR-99b). Possible functions of the miRNAs were assessed by use of the mirBase and DIANA TOOLS. Results indicated the expression of the 324 miRNAs, with 7 exclusivelly detected in high O2 environment (20%) and 5 in low O2 environment (5%). MiRNAS expressed in both environments, and with at least 2 Cts of difference between them, were investigated as potential regulators of oxidative stress response. We found 20 miRNAs more expressed in blastocysts cultivated in high O2 tension compared to low O2 tension. Pathways regulated by these miRNAs include FoxO signaling pathway (subclass O of Forkhead family of transcription factors) and protein processing in endoplasmic reticulum pathway, that are pathways associated to oxidative stress oxidative resistance and DNA repair. Changes in FoxO signaling pathway regulation were found mainly in high-tension culture system, with potential repercussions in cellular to proliferation and apoptosis. In addition, high-tension culture induced an increase in expression of miRNAs associated to the protein processing in endoplasmic reticulum pathway that might be related to stress response associated to protein folding failures. In conclusion, different culture atmospheres induce the differential expression of the miRNAs potentially associated to regulation of essential pathways to oxidative stress responses.

Financial support FAPESP (grants 2014/21034-3 and 2014/21042-6).

A217 Embryology, Developmental Biology and Physiology of Reproduction

Bacteriological, cytological and histopathological examination of the reproductive tract of slaughtered cows

J.B.S. Casarin[1], J.M. Trentin[2], M.F. Fiorenza[2], M.I.B. Rubin[1], A.P. Martini[1], T.S. Balestrin[1], G.A. Pessoa[1], S.S. de Barros[1], L.A.M. Centeno[1], G.M. Zanatta[1]

[1]Universidade Federal de Santa Maria, Santa Maria; [2]Universidade Federal do Rio Grande do Sul, Porto Alegre.

Keywords: endometritis, polymorphonuclear, uterine health.

Reproductive problems cause severe economic losses in dairy and beef herds (HUSZENICZA, 1999 Reproduction in Domestic Animals, v.34, p.237–245; MOSCUZZA, 2015 Turkish Journal of Veterinary and Animal Sciences, v.39, p.34-41). This study evaluated the efficiency of diagnostic methods applied to the reproductive tract of the bovine female. The presence of bacteria were measured (culture), the presence of polymorphonuclear cells (PMN) by cytology (Citobrush) secretion and lesion (macroscopic) in vagina, cervix and uterus; and microscopic lesions in the endometrium (uterine histopathology) on 23 reproductive tracts obtained after slaughter in abattoir. Statistical analysis was performed using Qui square test. In samples of the vaginal portion 8.69% (2/23) were turbid mucus secretion and 30.43% (7/23) crystalline mucus. Cytology 34.78% (8/23) showed the presence of PMN ≥ 10%. The culture showed 13.04% (3/23) of bacterial growth with Trueperella pyogenes 4.35% (1/23) and Escherichia coli 8.69% (2/23). In the vaginal portion, there was agreement between the bacteriological examinations vs cytology (P = 0.0019); and bacteriological vs macroscopic secretion (P = 0.000008). In the cervical portion 8.69% (2/23) of the samples were turbid mucus and 8.69% (2/23) crystalline mucus. Cytology revealed 13.04% (3/23) of the samples with PMN ≥ 10%. The bacterial culture revealed growth of Enterococcus faecalis in 4.35% (1/23) and gram negative oxidase positive in 4.35% (1/23) of the samples. In the uterus, 8.69% (2/23) had crystalline mucus, 4.35% (1/23) mucus bloody, 4.35% (1/23) purulent mucus and 4.35% (1/23) the uterus is presented hemorragic and edematous. In cytology, only one (1/23) positive sample for bacteriological examination (Staphylococcus epidermidis) and PMN. This same sample showed severe endometrial injury. Histopathological analysis showed 21.73% (5/23) with moderate injury endometrium and 17.39% (4/23) serious injury. There was agreement of bacteriological tests vs histological (P = 0.0006), histopatological vs macroscopic secretion (P = 0.0103) and histological vs cytological exam (P = 0.0001). There was no agreement of bacteriological and cytological exam(P = 0.3). However, there was a trend between the macroscopic vs citological (P = 0.06). It has been found that the association of culture, the secretion characteristics, cytology and histopatology are required for accurate diagnosis of the health of the reproductive tract. The detection of signs of inflammation, such as bloody discharge / purulent and inflammatory infiltrates did not correspond to the presence of bacterial culture, indicating an efficient immune response to contamination of the reproductive tract.

A218 Embryology, Developmental Biology and Physiology of Reproduction

Melatonin supplementation during *in vitro* maturation without gonadotropins restores embryonic development and reduces oxidative stress in bovine embryos *in vitro*-produced

P. Chediek Dall Acqua[1], M. Ambrogi[1], N.A.S. Rocha-Frigoni[2], B.C.S. Leão[2], L.T. Rodrigues[2], G.Z. Mingoti[3]

[1]Faculdade de Ciências Agrárias e Veterinárias, FCAV - UNESP, Jaboticabal; [2]Centro Universitário Católico Salesiano Auxilium - Unisalesiano, Araçatuba; [3]Laboratório de Fisiologia da Reprodução, Faculdade de Medicina Veterinária, Fmva - UNESP, Araçatuba.

Keywords: antioxidant, oocyte, reactive oxygen species.

The aim of this study was to evaluate the effects of melatonin (MT) in promoting the nuclear maturation and in the acquisition of competence for embryonic development in bovine oocytes matured *in vitro* in the absence of gonadotropins. In addition, we evaluated the antioxidant protection against reactive oxygen species (ROS) in oocytes and blastocysts. COCs (n = 807) were matured for 22h, at 38.5°C and 5% CO2 in air in 500 µL of B199 medium (TCM-199 with bicarbonate) with 10% FCS (Control without gonadotropins – CØ), supplemented with MT at 10^{-5}M (MT5), 10^{-7}M (MT7) or 10^{-9}M (MT9); the control group (C) was matured in B199 with 10% FCS, 0.5 mg/mL FSH and 100 IU/mL hCG. After IVM, a sample of oocytes was stained with 1 µg/mL Hoechst 33342 to assess the nuclear maturation. To determine the intracellular ROS levels, the matured oocytes were stained with 5 µM CellROX®Green (Molecular Probes, Eugene, OR, USA) and evaluated immediately under epifluorescence microscope. The images were analyzed by ImageJ software. The C group was chosen as a calibrator and the measured value of each treatment mean was divided by the mean of the calibrator to generate the relative fluorescence, expresses in arbitrary fluorescence units. Remaining oocytes were submitted to IVF (Day = 0) and the presumptive zygotes were cultured in SOF at 38.5°C and 5% CO_2 in air, for 7 days. The cleavage rates and embryonic development were evaluated at days 3 and 7 of IVC, respectively. The blastocysts were stained to evaluate intracellular ROS levels. Data were analyzed by ANOVA followed by Tukey's test ($P < 0.05$) and are presented as mean ± SEM. The absence of gonadotropins in association with different MT concentrations did not affect the nuclear maturation, as we found no differences ($P > 0.05$) in the metaphase II (MII) rates in comparison to C group (65.7 ± 8.0% to 73.6 ± 4.2%). The intracellular ROS levels in oocytes did not differ ($P > 0.05$) between treatments (0.8 ± 0.0 to 1.0 ± 0.0). The cleavage rates were lower ($P < 0.05$) in all the groups matured without gonadotropins (56.6 ± 7.4% to 59.8 ± 2.2%) compared to C (80.6 ± 2.6%). The blastocysts rates were higher ($P < 0.05$) in C (43.8 ± 2.6%) compared to MT5 (15.9 ± 3.7%) and CØ (28.5 ± 3.3%), but did not differ ($P > 0.05$) from MT7 (32.0 ± 2.9%) and MT9 (37.4 ± 2.2%). However, ROS amounts in blastocysts were higher ($P < 0.05$) in C (1.0 ± 0.1) than in MT9 (0.8 ± 0.1) and CØ (0.6 ± 0.0), but did not differ ($P > 0.05$) from MT5 (0.9 ± 0.1) and MT7 (0.8 ± 0.1). In conclusion, maturation of bovine oocytes in the absence of gonadotropins but in the presence of FCS and with different concentrations of MT did not affect MII rates. Although, MT did not promote any antioxidative effects in reducing ROS levels in oocytes, the addition of 10^{-9}M MT resulted in blastocyst rates similar to those obtained when oocytes were matured in the presence of gonadotropins and also reduced ROS levels in blastocysts.

Acknowledgments: FAPESP (#2013/07382-6) and CAPES.

A219 Embryology, Developmental Biology and Physiology of Reproduction

Effects of modulation of oxidative stress and lipid content on the developmental potential, quality and cryotolerance of *in vitro*-produced bovine embryos

G.B. Nunes[1], P. Chediek Dall Acqua[1], M. Ambrogi[1], B.C.S. Leão[2], N.A.S. Rocha-Frigoni[2], G.Z. Mingoti[1]

[1]Faculdade de Ciências Agrárias e Veterinárias, FCAV - UNESP, Jaboticabal; [2]Centro Universitário Católico Salesiano Auxilium, Unisalesiano, Araçatuba.

Keywords: antioxidants, lipolysis, cryopreservation.

The aim of this study was to evaluate the effects of supplementation with antioxidants during IVM and with stimulators of lipolysis (Forskolin: Fsk) and/or with inhibitor of lipogenesis (Conjugated Linoleic Acid: CLA) during IVC, on the developmental competence, quality (assessed by quantification of intracellular lipid content and reactive oxygen species - ROS) and cryotolerance of the in vitro-produced embryos. Oocytes (n = 1025) were matured in 500 µL of TCM-199 medium with bicarbonate, hormones and 10% FCS (IVM control), supplemented with a mixture of antioxidants (100 µM cisteamine, 0.6 mM cisteine and 100 UI catalase; IVM Atx) for 24h at 38.5ºC and 5% CO2. After IVF, presumptive zygotes were cultured in SOF medium with 0.5% BSA and 2.5% FSC (IVC control), supplemented with 100 µM CLA throughout all the IVC period (7 days; IVC CLA) and/or with 5 µM Fsk from day 6 of IVC (IVC Fsk). Thus, treatments were: IVM and IVC control (C); IVM Atx and IVC control (A); IVM Atx and IVC CLA (A+CLA); IVM Atx and IVC Fsk (A+Fsk); and IVM Atx and IVC CLA+Fsk (A+CLA+Fsk). The cleavage and embryonic development were evaluated on days 3 and 7 of IVC, respectively. Initial blastocysts (Bi) and blastocysts (Bl) were stained with 5 µM H2DCFDA (Molecular Probes, Invitrogen) to evaluate intracellular content of ROS, and with Sudan Black B 1% to evaluate the lipid content; they were immediately evaluated on an inverted epifluorescence microscope and on a light microscope, respectively. The images of stained embryos were analyzed by Q-Capture Pro Image Software and results were expressed as arbitrary units of intensities of pixels. Expanded blastocysts (Bx) were vitrified (Vitri-Ingá®, IngáMed®), thawed and cultured for 24h in SOF with 20%FSC to determine the re-expansion rates. Data were analyzed by ANOVA followed by Tukey's test and re-expansion rates by $\chi 2$ ($P < 0.05$). Cleavage (73.9 ± 6.1% to 83.5 ± 4.9%) and blastocysts rates (40.2 ± 3.1% to 49.6 ± 3.6%) did not differ between groups ($P > 0.05$), as well as the intracellular ROS content of embryos (1.00 ± 0.06 to 1.26 ± 0.09; $P > 0.05$). Lipid content was higher ($P < 0,05$) in C (1.02 ± 0.07) and A (1.02 ± 0.07) groups, compared with A+CLA (0.64 ± 0.04), A+Fsk (0.79 ± 0.02) and A+CLA+Fsk (0.73 ± 0.66). Re-expansion rate after warming and culture for 24h was higher ($P < 0.05$) in A+CLA (58.14%) and A+CLA+Fsk (67.65%) groups, compared with A+Fsk (35.42%); however, all these treatments were similar ($P > 0.05$) to C (55.88%) and A (45.45%) groups. In conclusion, there was no reduction in intracytoplasmic ROS content of embryos produced from matured oocytes in the presence of antioxidants; however, the supplementation of culture medium with CLA and Fsk, alone or combined, reduced the intracellular lipid content of such embryos. Treatment with CLA alone or combined with Fsk, improved the cryotolerance compared with Fsk treatment.

Acknowledgements: CAPES and FAPESP (#14/10288-4).

A220 Embryology, Developmental Biology and Physiology of Reproduction

Effects of supplement with sunflower seed on the production of bovine oocytes and embryos *in vitro*

A.L. Baltazar[1], S.M. Garcia[2], G.M. de Mattos[2], B.M. Ropelli[2], C. Castilho[2], J.O. Folino[3], G.C. Lupatini[1], G. Pugliesi[4], M. Binelli[4], C.M.B. Membrive[1]

[1]UNESP, Araçatuba, [2]UNOESTE, Presidente Prudente, [3]Quali's In Vitro, Presidente Prudente, [4]USP, Pirassununga.

Keywords: linoleic acid; omega 3; bovine.

Supplementation with compounds rich in linoleic acid, among which is included sunflower seed, promotes increase in conception rates in cows. It was hypothesized that sunflower seed supplementation in oocyte donors increases the number and quality of oocytes, increases the cleavage rates and determines an increase in the number and quality of blastocysts produced in vitro. Therefore the objective was to investigate the effect of such supplementation, in the number and quality of oocytes cultured in vitro on cleavage rate and in the number and quality of in vitro produced blastocysts. Therefore, five cows and twenty-five heifers (n = 30) Nellore were divided into two groups to receive one of the following treatments: 1.7 kg / day supplement containing 53% soybean meal 44% and 47% PB corn (Control Group - Group C, n = 15) or 1.7 kg / containing 40% add-on of soybean meal with 44% crude protein (CP) and 60% sunflower seed (Sunflower group - Group C; n = 15) for 57 days. Females underwent follicular aspiration on days (D) 0, 13, 29, 43, 56 and 77 (D0 = start of supplementation; D56 = end of supplementation). The oocytes were subjected to in vitro embryo production process. Data were analyzed by the Mixed procedure (SAS) version 9.2, by ANOVA mixed model. There was no difference between Groups C and G on the number of displayed follicles (16.85 ± 1.32 vs. 16.12 ± 1.48); number of aspired oocytes (13.80 ± 1.27 vs. 13.05 ± 1.25); recovery rate (82 ± 1% vs. 80 ± 2%); cleavage rate (76.32 ± 1.58% vs. 76.78 ± 2.11%); number of embryos (10.79 ± 0.94 vs. 10.08 ± 1.01); number of blastocysts (5.89 ± 0.66 vs. 4.48 ± 0.6); embryos number of Grade I and II (5.49 ± 0.64 vs. 4.17 ± 0.57); plasma concentrations of total cholesterol (85.76 ± 1.20 vs. 87.11 ± 1.18 mg / dl), triglycerides (14.98 ± 0.29 vs. 14.26 ± 0.27 mg / dL); HDL (35.12 ± 1.10 vs. 35.40 ± 1.0 mg / dL) and LDL (47.92 ± 1.58 vs. 48.85 ± 1.51 mg / dL), for the respective groups. It was concluded that sunflower seed supplementation in oocyte donors did not increase the number and quality of oocytes, cleavage rates and the number and quality of blastocysts produced in vitro.

Acknowledgements: CAPES, FUNDUNESP, Quali's in vitro Laboratory, PROGEST and Experimental of UNOESTE Farm.

A221 Embryology, Developmental Biology and Physiology of Reproduction

Pre-maturation of bovine oocytes with cAMP modulators and antioxidants during transport: effects on embryonic development and cryotolerance

M. Ambrogi[1], P. Chediek Dall Acqua[1], B.C.S. Leão[2], N.A.S. Rocha-Frigoni[2], G.B. Nunes[1], L.T. Rodrigues[2], G.Z. Mingoti[1]

[1]Faculdade de Ciências Agrárias e Veterinárias, FCAV - UNESP, Jaboticabal; [2]Centro Universitário Católico Salesiano Auxilium, Unisalesiano, Araçatuba.

Keywords: meiosis inhibitors, antioxidants, transport bovine oocyte.

The objective of this study was to evaluate the effects of supplementation with meiosis inhibitors and antioxidants during transport of bovine oocytes for 6 hours on the subsequent embryonic development and cryotolerance. Oocytes (n = 1.115) were transported in a portable incubator (Minitub®) for 6 hours in 400 mL of transport base medium (TBM: TCM-199 with 0.3% BSA and antibiotics) according with the following treatments: 1) Transp Contr group: transport in TBM; 2) Transp Contr+Atx group: transport in TBM supplemented with a mixture of antioxidants (100 mM cysteamine, 0.6 mM cysteine and 100 IU catalase); 3) Transp Pre-IVM group: transport in TBM supplemented with meiosis inhibitors (100 mM forskolin and 500 mM IBMX); and 4) Transp Pre-MIV+Atx group: transport in TBM supplemented with meiosis inhibitors and a mixture of antioxidants. After transport, the oocytes were matured in vitro for 18 hours (total duration of cultures = 24 hours) in 100 mL of IVM medium (TCM-199 supplemented with bicarbonate, hormones, and 10% FBS) in an incubator at 38.5°C and 5% CO2. One additional control group was included in the experiment (Contr group), in which the oocytes were matured in IVM medium, in an incubator for 24 hours. After fertilization, the presumptive zygotes were in vitro cultured in SOFaa 5 mg/mL BSA and 2.5% FBS for 7 days. The cleavage and blastocyst rates were evaluated, respectively, at days 3 and 7 (IVF = Day 0). At day 7, blastocysts were vitrified (Ingamed®, Maringa - PR, Brazil), and after warming, they were cultured for 24 hours to evaluate the re-expansion rates. The statistical model was a 2x2 factorial design, including the effect of the inhibitors in the transport medium, antioxidants and interactions. Means were compared by Tukey's test, including an additional control group (Contr). As there were no significant interactions ($P > 0.05$), only the main effects are demonstrated. The re-expansion rates were evaluated by Chi-squared test ($P < 0.05$). The cleavage and blastocyst rates were not affected ($P > 0.05$) by meiosis inhibitors (with inhibitors: 76.3 ± 1.6% and 38.3 ± 2.7%; without inhibitors: 81.0 ± 2.1% and 37.6 ± 2.8%; respectively) and they did not differ from Contr group (83.4 ± 2.7% and 39.8 ± 4.5%, respectively). Similarly, cleavage and blastocyst rates were not affected ($P > 0.05$) by antioxidants (with antioxidants: 76.8% ± 2.0 and 36.0 ± 2.6%, without antioxidants: 80.6 ± 1.6% and 39.9 ± 2.7%; respectively) and they did not differ from Contr group (83.4 ± 2.7% and 39.8 ± 4.5%, respectively). Embryo re-expansion after warming was similar ($P > 0.05$) between treatments (50.0% to 71.4%) In conclusion, the transport of bovine oocytes in medium supplemented with blockers of meiosis and/or with antioxidants during the period of 6 hours did not affect the in vitro production of embryos, as well as their cryotolerance. Further studies will be conducted to assess the maintenance of the quality of oocytes during longer transport periods.

A222 Embryology, Developmental Biology and Physiology of Reproduction

Effect of L-carnitine and *trans-10,cis-12* isomer CLA during culture in embryonic development and lipid content of bovine embryos produced *in vitro*

L.R.O. Dias[1], L.O. Leme[1], J.F.W. Sprícigo[2], M.A.N. Dode[3], I. Pivato[1]

[1]Universidade de Brasília, Brasília; [2]Instituto Federal Catarinense, Concórdia; [3]EMBRAPA Recursos Genéticos e Biotecnologia, Brasília.

Keywords: bovine, cryopreservation, embryo.

Cryopreservation of IVP embryos is affected by the concentrations high of lipid droplets that accumulate inside the blastomeres. Therefore, the partial removal of intracytoplasmic lipids by stimulating of lipolysis chemically or by decreasing the uptake or the synthesis of fatty acids by the cells, can be an alternative to improve cryopreservation of IVP embryos. Then this study aimed to evaluate the effect of delipidant agents, L-carnitine and the *trans-10, cis-12* isomer CLA, during culturein the quantity, quality and lipid content of bovine embryos produced *in vitro*. A total of 2,448 1 and 2 grade oocytes obtained from slaughterhouse ovaries were matured *in vitro* for 24 hours at 38.5 ° C, 5% CO2. After the co-incubation of sperm and COC (16-18 hours), the presumptive zygotes were distributed into four treatments: T1) Control (n = 616): SOFmedium supplemented with 5% FCS (Invitrogen, Carlsbad, California, USA); T2) L-carnitine (n = 648): SOF medium supplemented with 5% FCS plus 0.6 mg ml-1 of L-carnitine (Sigma, St. Louis, Missouri, USA); T3) CLA (conjugated linoleic acid) (n = 627): SOF medium supplemented with 5% FCS plus 100 uM *trans-10 cis 12 CLA* (Sigma, St. Louis, Missouri, USA); and T4) L-carnitine + CLA (n = 597): SOF medium supplemented with 5% FCS plus 0.6 ml-1 mg of L-carnitine and 100 uM of *trans-10 cis-12* CLA. The cleavage and blastocyst rates were evaluated on D2 and D7 of culture, and expanded blastocysts (Bx) were stored for lipid quantification by Sudan Black B stain (Sudano et al. 2012. Biology of Reproduction, v.87, p .1-11). Embryo production data were analyzed by Chi-square test ($P < 0.05$) and lipids quantification by analysis of variance (ANOVA) ($P < 0.05$). Cleavage rate was similar ($P > 0.05$) among all treatments (T1 = 95 ± 4.3; T2 = 95 ± 3.5; T3 = 95 ± 3.7 e T4 = 95 ± 3.1). Blastocyst rate was higher ($P < 0.05$) on the control group than the other treatments, wich were similar ($P > 0.05$) among on D6 (T1 = 19 ± 2.7; T2 = 13 ± 2.4; T3 = 14 ± 2.6 and T4 = 13 ± 2.6) and on D7 of culture (T1 = 49 ± 3.5; T2 = 39 ± 3.0; T3 = 42 ± 3.9 and T4 = 39 ± 3.9). There was no difference between treatments ($P > 0.05$) on the speed of development, and in all treatments the majority of D7 embryos were already expanded blastocyst. Embryos from T2 showed lower cytoplasm lipids than those from T1 ($P = 0.0138$) and T3 ($P = 0.0261$), being T4 similar to all treatments. The results suggested that supplementation with delipidant agents had not affected quality but had negatively affected embryo production. However, the presence of L-carnitine during CIV decreased the amount of lipids, suggesting that its use can result in bovine IVP embryos more resistant to cryopreservation.

A223 Embryology, Developmental Biology and Physiology of Reproduction

Low-oxygen compared with high-oxygen atmosphere in bovine parthenogenetic embryo culture

C.F.G. de Jesus, L.C.B. Motta, D.Í.A. Teixeira, L.M. Melo, M.H. Bhat, V.J.F. Freitas

UECE, Fortaleza.

Keywords: bovine, parthenogenesis, embryo culture.

During embryo culture, the atmosphere of traditional 5% carbon dioxide (CO2) in air (approximately 20% oxygen [O2]) has a higher O2 concentration than it is physiologically normal in the oviduct and uterus. It has been claimed that this promotes the generation of cytotoxic highly reactive oxygen radicals detrimental to the embryo. Thus, the aim of this study was to compare parthenogenetic embryo culture in 5% CO2 in air (two gases) with 5% CO2, 5% O2, and 90% N2 (three gases). The ovaries used for obtaining cumulus-oocyte complexes (COCs) were originated from local slaughterhouse. The COCs were selected and then in vitro matured in TCM-199 supplemented with 10% FCS, under mineral oil, and placed in incubator with 5% CO2 in air, at 38.5°C for 26 h. After maturation, COCs were denuded in TCM-199 with hyaluronidase for 3 min in vortex. A total of 472 oocytes were chemically activated using 5 μM ionomycin, for 5 min, and 2 mM 6-DMAP, for 6 h. Posteriorly, presumptive embryos were randomly divided into two groups: two (2G) or three gases (3G) culture. The 2G embryos (n = 237) were cultured in incubator (Thermo 3110, Thermo Fisher Sci., Marietta, USA) with 5% CO2 in air, at 38.5°C, while 3G embryos (n = 235) were cultured in mini-incubator (Eve, WTA Tec. Apl., Cravinhos, Brazil) with 5% CO2, 5% O2, and 90% N2 at 38.5°C. The culture was performed for nine days in SOF medium with 5% FCS. In both groups, at the third day of culture, 2% B-27 was added to the culture medium. The embryo production was observed at three, seven and nine days of culture to verify cleavage, blastocyst and hatching rate. The percentage were compared using Fisher's exact test, considering significant when $P < 0.05$. The cleavage rate was higher ($P < 0.05$) in 2G (92.8%) than 3G group (86.0%). The blastocyst rate was similar ($P > 0.05$) between groups: 28.7% (2G) and 31.1% (3G). Concerning the hatching rate, the higher values ($P < 0.05$) were observed in 3G (14.9%) when compared to 2G group (5.1%). In conclusion, the final development of bovine parthenogenetic embryos had a positive effect when cultured in low-oxygen atmosphere.

A224 Embryology, Developmental Biology and Physiology of Reproduction

Does the addition of cow serum during the recent post partum period influence the initial embryonic development?

L.F. Collares[1], J.A.A. Rincón[1], B. Mion[1], P.C. Gindri[2], M.A. Borges[1], J.F. da Silva[1], J. Pradieé[1], L.M.C. Pegoraro[3], A. Schneider[1], M.N. Correa[1]

[1]Universidade Federal de Pelotas, Pelotas; [2]Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Ijuí; [3]EMBRAPA Clima Temperado, Pelotas.

Keywords: lactation, maturation, production in vitro.

The transition period of dairy cows, which is characterized by multiple metabolic disorders, extends from the last three weeks of gestation until the first three weeks of lactation. During this period, the food intake is not sufficient as to supply all the nutritional needs, which will be enhanced, leading to negative energetic balance (NEB). It is known that this NEB holds direct influence over fertility, affecting in a negative way the oocyte maturation, the "cumulus" cells expansion, and consequently the initial embryonic development. The objective of this study was to evaluate the effect produced by the addition of dairy cows blood serum in the maturation environment during the recent post partum and the end of lactation periods over the initial embryonic development. Bovine ovaries coming from local slaughterhouses, were collected and aspirated. The oocyte cumulus complexes (COCs) were randomly divided into three treatments groups of 50 COCs each: T0: ovine serum as control, T1: 10.8+-4.8 days-post partum cows serum, T2: 354+-3.8 days-post partum cows serum. Later, the COCs were incubated on at 39°C with 5% CO2 for 24 hours in the maturation medium (TCM 199 Gibco®, LH/FSH, pyruvate and antibiotics) enriched with serum (10%). The insemination was proceeded with a concentration of 1x106 at spermatozoon/ml using gradient Mini-Percoll® for the spermatic selection. After the insemination, the COCs were incubated for 18 hours. The zygotes remained in the SOFaa medium supplemented with ovine serum (5%) and pyruvate (0,33mmol) in the same conditions as the MIV for 7 days. The day of the insemination was considered as day 0, the cleavage rate was assessed on day 3 and the global embryonic development rate (blastocysts/number of inseminated) on day 7. According to preliminary results of three routines, the cleavage rate was lower than T1 at T0 and T2 ($P < 0.001$) the percentage of cleavage of T0 was of 81.6+-14.0%, of T1: 44+-12.1% and of T2: 70+-9.8.The embryonic development rate (blastocysts/inseminated) on T0 was of 16,0%+-5.5, T1: 21%+-2.92, and T2:16,0% +-13.0. From this data it can be suggested that the addition of cows serum during the recent post partum in the maturation medium showed a lower cleavage rate and similar embryonic development, compared to the other treatments. Overall, a bigger number of routines and complementary analysis are necessary to better understanding of the effects of supplementation with cow serum during the post partum over the bovine oocyte's maturation in vitro and later initial embryonic development.

A225 Embryology, Developmental Biology and Physiology of Reproduction

Embryo development after transfer into the oviduct ipsi or contralateral to corpus luteum in sheep

V. de Brun[1], A. Meikle[1], F. Cuadro[2], N. Barrera[2], P.C. dos Santos-Neto[2], D. Bosolasco[2], M. Crispo[3], A. Menchaca[2]

[1]Facultad de Veterinaria, Montevideo; [2]Instituto de Reproducción Animal Uruguay, Montevideo; [3]Instituto Pasteur de Montevideo, Montevideo.

Keywords: embryo transfer, oviduct, CL.

The aim of this study was to investigate the embryo survival and development by transferring embryos on Day 1 after fertilization to the ipsi or contralateral oviduct to corpus luteum (CL). Twenty multiparous Corriedale ewes received an estrus synchronization protocol that consisted in the insertion of an intravaginal progestagen sponge (medroxyprogesterone 60 mg, Progespon, Syntex, Argentina) during 7 days associated with 200 IU of eCG (Novormon, Syntex) and 125 mg of prostaglandin F2alpha analog (cloprostenol, Ciclase DL, Syntex) at sponge removal. The occurrence of estrus was checked using vasectomized rams from 24 to 48 h after sponge removal. This protocol synchronizes the ovulation around 60h after sponge removal (Menchaca and Rubianes, 2004). Four hundred and ninety nine (n = 499) in vitro produced zygotes were transferred into the oviduct of 13 recipient ewes (17-20 embryos per oviduct ipsi and contralateral to CL; 35-40 embryos per ewe) on Day 1 after in vitro fertilization (IVF)/ovulation (Day 0: IVF for the embryos, and 60 h after sponge removal for the recipients). Only those recipients that showed estrus and ovulation from only one ovary (checked by laparoscopy previous to embryo transfer) were used. Embryo transfer was performed through the fimbria into each oviduct with the zygotes uploaded in a tomcat with 20 ug of holding medium. Five days later (Day 6) the embryos were collected by flushing from each uterine horn to be evaluated for stage and quality according to the IETS recommendations. Statistical analysis was performed by using chi square test. The recovery rate (embryos collected/transferred) was higher in the ipsi than in the contralateral uterine horn respect to the CL (43.6% 109/250 vs. 30.1% 75/249, $P < 0.05$), and the proportion of viable embryos (Grade 1 to 3) was also greater in the ipsilateral side (23.6% 59/250 vs. 15.3% 38/249, respectively; $P < 0.05$). In addition, lower proportion of degenerated embryos/collected ova and embryos was obtained when the embryos were transferred into the ipsi vs. contralateral oviduct (22.9% 25/109 vs. 42.7% 32/75 respectively, $P < 0.05$). Differences in embryo development (blastocysts/total ova and embryos) did not reach significance (23.9% 26/109 vs. 14.7% 11/75 respectively, $P = 0.15$). In conclusion, greater recovery rate, better embryo development, and less degenerated embryos were obtained when transferring zygotes to the ipsi than the contralateral oviduct on Day 1 after fertilization/ovulation. We suggest a local effect of the CL on the ipsilateral oviductal environment affecting early embryo development.

A226 Embryology, Developmental Biology and Physiology of Reproduction

Effects of SNAP and/or cilostamide supplementation during bovine oocyte *in vitro* maturation on maturation kinects and embryo production

R.C. Botigelli[1], K.L. Schwarz[2], M. Del Collado[2], F.C. Castro[2], H. Fernandes[2], C.L.V. Leal[2]

[1]IB-UNESP Botucatu-Farmacologia, Pirassununga; [2]Faculdade de Zootecnia e Engenharia de Alimentos - FZEA/USP, Pirassununga.

Keywords: Nitric oxide, phosphodiesterases, IVP.

Nitric oxide (NO) is a gaseous free radical involved in many physiological processes in mammals, which has been detected in ovaries, oocytes and embryos. NO activates soluble guanylate cyclase (sGC), and results in the production of cyclic guanosine monophosphate (cGMP). This nucleotide is involved in oocyte maturation and consequently in the success of fertilization. In addition to cGMP, another cyclic nucleotide, cyclic adenosine monophosphate (cAMP), is also related to maturation. The levels of these nucleotides are balanced by synthesis and degradation, made by phosphodiesterases (PDEs). The aim of this study was to analyze the effect of NO donor (SNAP) and PDE3 inhibitor (cilostamide) during IVM on maturation kinects in bovine oocytes and in vitro embryo production. Experiment I, cumulus-oocyte complexes (COCs) were cultured in maturation medium with a NO donor (0.1 μM S-nitroso-N-acetylpenicillamine - SNAP) associated or not with a PDE3 inhibitor (20 μM cilostamide) at 28h and after this period, the maturation kinects was evaluted. In Experiment II, COCs were cultured for 28 h in maturation medium with NO donor (0.1 μM SNAP) or PDE3 inhibitor (20 μM cilostamide), or both, after this period the COCs were submited to IVF and IVC, the developmental rates was evaluated. Statistical analyses were performed using the SAS System. Data were tested for normal distribution and transformed to arcsine. The percentages of maturation rates and embryo production were analyzed by one-way ANOVA followed by Bonferroni post hoc test). In experiment I, SNAP+cilostamide had lower MII rates at 24 h IVM (50.0 ± 2.0%, $P < 0.05$), but control and SNAP groups had similar proportions of mature oocytes (69.8 ± 5.5 and 67.1 ± 7.4%, $P > 0.05$), but at 28 h of IVM all groups were similar (66.6 to 71.4%, $P > 0.05$). In experiment II, cleavage rates were lower in the SNAP+cilostamide association (55.1 ± 7.6%, $P < 0.05$) compared to all other treatments, which were similar to each other (72 to 74%, $P > 0.05$). Blastocyst rates on D7 were similar for control, SNAP and cilostamide (38.7 ± 5.8, 37.9 ± 6.2 and 40.5 ± 5.8 %, respectively, $P > 0.05$), but lower for SNAP+cilostamide (25.7 ± 6.9%, $P < 0.05$). Similar trend was observed for D8 blastocyst and hatching rates, where SNAP+cilostamide had lowest rates for both parameters ($P < 0.05$). In conclusion, delaying meiosis by combining SNAP and cilostamide decreased embryos production.

A227 Embryology, Developmental Biology and Physiology of Reproduction

Changing maternal nutrition in early pregnancy modifies fetal ovary transcriptome in Nelore cow

H.F. da Costa[1], M.C.V. Miguel[2], A.M. Pedroso[3], F.L. Lopes[2], J.R. Peiró[2], G.P. Nogueira[2]

[1]Laboratório Biovet, Vargem Grande Paulista; [2]FMVA-UNESP, Araçatuba; [3]Cargil Nutrição Animal, Piracicaba.

Keywords: nutrition, ovaries, oocytes.

Environmental influences such as nutritional restriction during early cows gestation may impair fetal development and compromise functions in its adulthood. During the first trimester of gestation fetal gonads are formed, is a critical period in fetal ovarian development, as oocytes differentiation, and need to escape from degenerative processes to remain in the ovaries. We hypothesized that either restriction or excess of nutrients ingested by cows during the first days of pregnancy interferes in fetal body and ovarian weight and transcriptome. Twenty-one uniparous Nelore cows (BW = 488 ± 24 kg, BSC = 3.1 ± 0.1) were subjected to Timed Artificial Insemination with sexed semen (female) of a single bull and individually allocated on different diets. The diet of the control group (C) met the maintenance requirements, and the groups of high (A) and low (B) either 180% or 60% of maintenance respectively. Live weight and body condition score were assessed weekly to adjust the diet according to individual weight of each animal. At 60 days of gestation, eight fetuses were removed by colpotomy (accessed trough vagina), ovaries were dissected and weighed. One fetal ovary (of each pair) was sequenced (pair-end reads – TruSeq RNA Sample PrepGuide, HiScan 500 – Ilumina, Inc., San Diego, CA, EUA). The samples mapped with Bos taurus genome (UMD3.1, masked version). The effect of treatments on both ovarian and fetal weight was compared by ANOVA (SAS 9.3, proc GLM). The fetus ovary weight (sum of the two) was lower in group B ($P < 0.04$, 0.007 ± 0.001g) than in both Groups A (0.02 ± 0.004g) and C (0.013 ± 0.007g) that did not differ ($P > 0.08$) between treatments. The fetal weight in group B was lower ($P < 0.006$; 12.8 ± 1.14 g) than in groups A (20.56 ± 2.2 g) and C (20.03 ± 0.8g). The maternal nutritional status during the first 60 days of gestation changed the transcriptome of fetal ovaries. There were differences in the pattern of gene expression between the control groups, high and low intakes. A total of 79 genes out of 20,657 showed differential expression between treatments (FDR 0.05) some of them related to embryonic and ovarian development. Thus, we can conclude that changing the maternal nutrition during the first 60 days of gestation will change the transcriptomic profile of fetal ovaries. The mother's poor nutrition jeopardizes ovarian size and weight and fetal weight suggesting impairment on the production of ovarian follicles. Thus, the development impairment at the beginning of meiosis could reduce the number of oocytes in the females ovary.

Acknowledgements: FAPESP 2011/50839-1; CNPq 487036/2013-3, CAPES.

A228 Embryology, Developmental Biology and Physiology of Reproduction

Effects of cAMP modulators during *in vitro* prematuration of bovine oocytes on gap junctional communication and embryo development potential

N.Z. Saraiva[1], C.S. Oliveira[2], M. Del Collado[3], M.R. Lima[4], S.C. Méo[5], M.R. Figueiró[1], R. Vantini[4], J.M. Garcia[4]

[1]EMBRAPA Amazônia Oriental, Belém; [2]EMBRAPA Gado de Leite, Valença; [3]FZEA – USP, Pirassununga; [4]FCAV - UNESP, Jaboticabal; [5]EMBRAPA Pecuária Sudeste, São Carlos.

Keywords: prematuration, cAMP modulators, oocyte competence.

In vitro oocyte maturation is fundamentally limited by the quality of the oocyte, namely by the intrinsic developmental competence of this structure. This study aimed to evaluate the effects of the addition of cAMP modulators during IVM of bovine oocytes in the SPOM system (simulated physiological oocyte maturation) by checking the status of communication between oocytes and cumulus cells through gap junctional communication (GJC), and early embryonic development from structures subjected to these modulators, seeking positive impact of this system on oocyte competence. Oocytes were *in vitro* matured in TCM 199 supplemented with 10% FBS, and subjected in treated groups to 100 µM or 150 µM forskolin and 750 µM IBMX for the first 2 h of culture. Subsequently, oocytes were transferred to base medium supplemented with 20 µM cilostamide. To evaluate the level of connection between the oocyte and the cumulus cells, it was measured the transfer of dye calcein AM for GJC, according to the protocol described by Thomas et al. (Biol. Reprod. 70, 548-556). The emission of intra-oocyte fluorescence was measured with a fluorescence microscope aid, and the images captured by camera and analyzed in ImageJ software. Oocytes from the same treatments were also submitted to IVF procedure in TALP medium and CIV in SOFaa with 6 mg/mL BSA and 2.5% FBS, evaluating early embryonic development. Analyses were performed in GraphPad INSTAT 6.01, and the fluorescence results were submitted to ANOVA and means were compared by Tukey test, and proportions of cleaved embryos and blastocysts evaluated by chi-square test (χ^2). IVM process caused a significant increase ($P < 0.05$) in the GJC fluorescence intensity in all groups, with the superior means in the groups treated with cAMP modulators. There was no difference between groups 100/750 and 150/750. In relation to preimplantation development, we found that the treatments affected negatively the cleavage rates (control - 585/677 (86.4)^a; 100/750 - 542/663 (81.7)^b; 150/750 - 558/688 (81.1)^b). When the proportion of blastocysts (D8) was calculated by the total number of oocytes, we verified no significant differences between the groups (35.2 to 37.9%). Nonetheless, when the proportions were calculated from the cleaved embryos, we observed superiority of groups 100/750 (53,3)^a and 150/750 (48,8%)^a in comparison to the control group (38.9%)^b. Thus, we conclude that the use of cAMP modulators during IVM causes inhibitory or retarding effect on the first cleavages; however, stimulatory effect in those structures which overcome the initial block phase. Moreover, cAMP modulators caused considerable increase of GJC during IVM.

Financial Support: FAPESP 2010/20744-6 and 2011/12983-3.

A229 Embryology, Developmental Biology and Physiology of Reproduction

Histology and morphometry of uterine horns of nulliparous and multiparous bitches

M.P. Lourencini[1], J.L.G. Ramos[2], J.D. Santos[1], L.M. Sena[1], L.L. Oliveira[1], C.B. Martins[1]

[1]Universidade Federal do Espirito Santo, Alegre; [2]Universidade Norte Fluminense Darcy Ribeiro, Campos.

Keywords: canine, histomorphometry, uterus.

There are several studies on the histological features in the different phases of the estrous cycle in dogs, however, there exists few publications about the changes in the female reproductive system related to post gestational uterine involution, its mechanisms and whether there is an effective response, especially uterine, as to its shape, size, volume and re-epithelialization. It is known that the uterus is composed of three main layers: the endometrium, myometrium and perimetrium and all these layers undergo changes during pregnancy to allow fetal growth and development. Due to successive pregnancies, many uterine pathologies occur mainly by changes in these layers, as myometrial relaxation, glandular hyperplasia, irregular endometrial and uterine vessels sclerosis (AUGSBURGER; KURZI, 2004; MONTEIRO et al., 2009). Based on limited information and research on the possible changes found in uterine horns of nulliparous and multiparous bitches, this study aimed to evaluate the presence of histologic and morphometric changes that may occur in this organ between the aforementioned groups. We evaluated the right and left uterine horns of 13 dogs of different breeds and ages, 7 nulliparous and 6 multiparous. After ovariohysterectomy fragments approximately 0.5 cm long were collected individually from the cranial, medial and caudal parts (in relation to the ovary) of each uterine horn, for histological and morphometric evaluation of the total thickness of the uterine wall (μm), total endometrium (μm), diameter of the endometrial glands (μm) and height of the glandular epithelium (μm), total myometrium thickness (μm), internal and external myometrium and vascular stratum (μm). According to the evaluation of histological sections of ovaries and uterus , it was possible to estimate the phase of the reproductive cycle in which animals were by visualization of these structures in the ovaries , thickness and characteristics of the uterine horns in addition to reproductive history described by owners. Thus, it was found that nine animals were in diestrus, two and two in estrus in proestrus. The means between the two groups were compared by Student's t-test at 5% significancy. The measured structures didn't show significant differences except in internal myometrium in the left caudal medial and right caudal segment which may have been influenced by the phase of the reproductive cycle of these animals. Measured structures showed no significant differences. Given the results obtained in this study it can be concluded that the uterine horns of nulliparous and multiparous bitches are similar histologically and histomorphometrically. The study was approved by the Ethics Committee for Animal Use in Experimentation (CEUA), the Federal University of Espírito Santo (UFES), Alegre campus, protocol number 052/2014.

A230 Embryology, Developmental Biology and Physiology of Reproduction

Effect of various concentrations of folic acid on *in vitro* culture of bovine embryos

A.L.S. Guimarães[1], M.A.N. Dode[2]

[1]Universidade de Brasilia, Brasília; [2]EMBRAPA Recursos Genéticos e Biotecnologia, Brasília.

Keywords: blastocyst, antioxidants, embryo development.

Reactive oxygen species (ROS) are accumulated during embryo development in response to oxidative stress that occurs during in vitro culture. The presence of intracellular ROS can induce apoptosis and, consequently, compromises embryo development. Studies have shown that antioxidant supplementation of culture medium can reduce the stress that embryos are exposed (Kim et al, Mol Rep Dev. 76: 120-125 2009; Koyama et al, Rep Dom Anim. 47: 921-927 2012). Considering that folic acid is present in follicular and uterine environment in mammals, and that it has antioxidant properties, we hypothesized that it has a positive effect on embryo and can improve IVP results. This study aimed to evaluate the supplementation of different concentrations of folic acid on in vitro culture of bovine embryos and its effect on developmental kinetics. COCs were aspirated from slaughterhouse ovaries and after selection were matured for 24 h, and subsequently, were co-incubated with the sperm for 18 h. After fertilization period, presumptive zygotes were distributed into 5 groups: T1 (Control), SOF (N = 257); T2 (10μM), SOF with 10μM of folic acid (N = 239); T3 (20 μM), SOF with 20 μM of folic acid (N = 242); T4 (50 μM), SOF with 50 μM of folic acid (N = 253) and T5 (500 μM), SOF with 500 μM of folic acid (N = 243). In all groups the SOF medium was supplemented with 0.4% BSA. Embryonic development was evaluated on D2 for cleavage and on D6 and D7 for blastocyst rate, which were categorized in Bi, Bl, Bx and BE. Data were evaluated by Chi-square test ($P < 0.05$). Cleavage rate was lower in group D2 500 μM (74.9%) than in the other groups (T1: 82.9%; 10 μM: 79.1%; 20 μM: 83.1%; 50 μM: 79.1%). Similarly, the group 500 μM showed lower rate ($P < 0.05$) of blastocyst on D6 when compared to the other groups (T1: 11.3%; 10 μM: 14.7%; 20 μM: 14.4%; 50 μM: 13%; 500 μM: 7.8%). However, no differences among treatments in the blastocyst rate were detected on D7 (T1: 35.4%; 10 μM: 33.5%; 20 μM: 36%; 50 μM: 30.4%; 500 μM: 29.6 %). In D6 percentage of embryos that were at BX stage was higher ($P < 0.05$) on 10 μM (25.7%) and 20 μM (23.5%) groups than in T1 (6.8 %) and 500 μM (0%) groups; the 50 μM group (12.2%) was similar to all treatments. However, the D7 BX rate was similar ($P > 0.05$) among all treatments (T1: 44.0%; 10 μM: 47.5%; 20 μM: 42.5%; 50 μM: 37, 6%; 500 μM: 36.1%). The results suggest that supplementation of 10 μM to 20 μM of folic acid during in vitro culture did not affect the production of embryos, but accelerated the stage of development at D6. To confirm the beneficial effect of folic acid on the embryo quality, additional assessments are required.

A231 Embryology, Developmental Biology and Physiology of Reproduction

Modulation of *in vitro* bovine embryos by t2iGöLIF increases the number of Nanog expressing cells in the ICM but does not prevent the activation of Sox17

J.R. Canizo[1], A.E. Ynsaurralde Rivolta[2], P. Fisher[3], M. Suvá[4], A.S. Guberman[5], J.F. Aller Atucha[6], D. Salamone[4], R.H. Alberio[7], R. Alberio[3]

[1]Facultad de Ciencias Exactas y Naturales.Universidad de Buenos Aires, Inta Eea Balcarce, Buenos Aires; [2]Facultad de Agronomía. Universidad de Buenos Aires, INTA Eea Mercedes Corrientes, Buenos Aires; [3]School of Biosciences, University of Nottingham, Nottingham; [4]Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires; [5]Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires., Buenos Aires; [6]INTA Eea Balcarce, Balcarce; [7]INTA Eea Balcarce-Universidad de Mar Del Plata, Balcarce.

Keywords: pluripotency, early bovine embryogenesis, modulation.

The combination of inhibitors known as 2i (MAPK and GSK3b inhibitors) + LIF is used for culturing mouse naïve embryonic stem cells (ESC), the only type of cells able to colonize the germline efficiently. In human ESC 2i+LIF is not sufficient to confer naïve properties, but with the addition of a PKC inhibitor (Gö6983) and a modified concentration of GSK3B inhibitor (known as t2iGöLIF), it is possible to capture naïve pluripotency in newly derived cell lines. In this study we analysed the effect of t2iGöLIF treatment during early bovine embryogenesis. Bovine embryos were produced by IVF and cultured in vitro in serum free medium BBH7 until day 5 and then in N2B27 medium with 20 ng/ml of h-LIF or t2iGö, t2iGöLIF and DMSO (control). Embryos fixed at day 8 were analysed by immunocytochemistry for Nanog and Sox17, epiblast and hypoblast markers, respectively. Comparison of blastocyst development showed no significant difference between control medium with DMSO (0.24 ± 0.05) vs. LIF (0.22 ± 0.04) and t2iGöLIF (0.32 ± 0.03), however t2iGö resulted in decreased embryo production (0.15 ± 0.03). After immunostaining we found that all treatments produced an increased inner cell mass (ICM) to trophectoderm (TE) ratio, ICM:TE were 0.25 ± 0.05 vs 0.39 ± 0.08, 0.50 ± 0.08 and 0.38 ± 0.08 (control vs t2iGö, LIF and t2iGöLIF, respectively). In all treatments we detected more Nanog positive cells than control (25 ± 4) vs. t2iGö (40 ± 7), LIF (44 ± 7 A) and t2iGöLIF (45 ± 8). The number of Sox17 positive cells was reduced in t2iGöLIF (19 ± 3) and was unchanged in t2iGö (38 ± 5) compared to control (34 ± 5). Surprisingly, more Sox17 positive cells were found in LIF treatment (61 ± 8) indicating that not only Nanog, but Sox17 increased with LIF. Furthermore, the number of cells that co-expressed both markers was higher in LIF treated groups (13 ± 4 vs 4 ± 1 in control). In conclusion, the results indicate that t2iGöLIF is the only treatment that reduces the number of primitive endoderm cells (hypoblast). These results suggest that additional signalling pathways may need to be blocked to prevent the differentiation of the early epiblast in cattle.

A232 Embryology, Developmental Biology and Physiology of Reproduction

Improvement of blastocyst rates and gene expression pattern of ICSI bovine embryos by IVM with high cysteamine concentration and the use of sperm previously incubated with COC's

N.G. Canel[1], R.J. Bevacqua[1], M. Suvá[1], M.E. Arias[2], R. Felmer[2], D. Salamone[1]

[1]laboratorio de Biotecnología Animal, Facultad de Agronomía, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires; [2]2laboratorio de Reproducción, Centro de Biotecnología de La Reproducción (CEBIOR-BIOREN), Universidad, Temuco.

Keywords: ICSI, bovine, gene expression.

In bovine, ICSI remains inefficient partially due to low levels of sperm decondensation. The addition of cysteamine (Cys) to the maturation medium was shown to increase the reduced glutathione (GSH) levels in mature oocytes, which acts synergistically with heparin (Hep) to promote sperm decondensation after fertilization (Romanato et al. Hum Reprod, 18:1868-73, 2003). On the other hand, cumulus cells induce sperm capacitation in mouse (Shimada et al. Am J Reprod Immunol. 69:168-79, 2013). The aim of this work was to assess the use of high Cys concentration during in vitro maturation, the incubation of sperm with COCs prior to ICSI, and the Hep supplementation to the culture medium to improve ICSI in bovine. Methods: COC's from slaughtered cows ovaries were matured for 21 h with 1 (1 mM groups) or 0.1 mM Cys (0.1 mM groups, standard condition). COCs were incubated for at least 3 h with 16x106 sperm/ml in Bracket's defined medium. Sperm attached to cumulus cells were used for ICSI (Inc groups). Sham (injected without sperm) and diploid parthenogenetic (PA) controls were performed. Oocytes were activated with 5 µM ionomycin (4 min), resting on TCM-199 for 3 h and 2 mM DMAP for another 3 h. PA group was treated with DMAP immediately after ionomycin. Embryos were cultured in SOF medium. One ICSI group was cultured in SOF with 1 mg/ml heparin for the first 48 h (1 mM+Hep group). Cleavage and blastocyst rates were evaluated on Days 2 and 7 of in vitro culture, respectively. For quality analysis of blastocysts, the relative abundance of mRNA coding for 8 genes was compared between 0.1 mM, 1 mM, and 1 mM-Inc ICSI groups by qPCR. An IVF control was included, following Brackett and Oliphant protocol. Differences among treatments were determined by Fisher′s exact test for cleavage and blastocyst rates and by one-way ANOVA and the LSD test for mRNAs relative quantification ($p \leq 0.05$). Results: 0.1 mM-Inc cleavage rates (n = 117, 92%) did not differ from Sham (n = 106, 89%) and PA control (n = 144, 96%) and were higher than 1 mM-Inc (n = 116, 79%). Lower cleavage rates were observed for 0.1 mM (n = 132, 60%), 1 mM (n = 108, 52%) and 1 mM-Hep (n = 114, 65%). However, 1 mM-Inc showed higher blastocyst rates than all other ICSI groups (23 vs. 11, 18, 11 and 14% for 1 mM-Hep, 1 mM, 0.1 mM-Inc and 0.1 mM, respectively). While PA control showed the highest blastocyst rates (61%), Sham control did not differ from any of the ICSI groups (21%). No differences were found for the relative abundance of mRNAs coding for INFτ, CAT, DNMT1, OCT4, and HDAC3 between all groups. SOD2, HADC1 and HADC2 expression was higher for 0.1 mM ICSI than for IVF embryos. ICSI 1 mM did not differ from IVF group for those 3 genes, neither did 1 mM-Inc, except for HDAC1. In conclusion, while the use of Hep during in vitro culture does not have a beneficial effect on embryo development, the use of 1 mM Cys during maturation and the sperm incubated with mature COCs, might be a good strategy to improve the ICSI outcomes in cattle.

A233 Embryology, Developmental Biology and Physiology of Reproduction

Morphological and functional development of the corpus luteum of Holstein cows treated with ECG before or after ovulation

B.M. Monteiro[1], P.L.J. Monteiro Junior[2], L.H. Oliveira[2], A.B. Prata[2], J.N. Drum[2], C. Spies[2], M. Binelli[1], R. Sartori[2], P.C. Papa[3]

[1]VRA-FMVZ/USP, Sao Paulo; [2]DZ-ESALQ/USP, Sao Paulo; [3]VCI-FMVZ/USP, Sao Paulo.

Keywords: gonadotropins, luteinization, progesterone.

This research aimed to compare the morphological and functional changes of the corpus luteum (CL) of cows treated with eCG before or after ovulation. Eighteen adult and dry Holstein cows were used. At random day of the estrous cycle (D-10) 2 intravaginal P4 (1.9 g) device (iP4, CIDR®, Zoetis, São Paulo, SP, Brazil) and intramuscular (im) 2 mg estradiol benzoate (EB, Gonadiol®) were used. Seven days after, on D-3, it was administered 25 mg of PGF2α (Lutalyse®). At this moment, the animals were divided into 3 groups: 6 cows that received no treatment (Control group); 6 cows received 400IU eCG (Novormon®) i.m. (D-3, pre-eCG group); or 6 cows who received 400IU eCG i.m. 3 days after estrus (D+3, post-eCG group). On D-2, the iP4 were removed and animals received 1.0 mg of estradiol cypionate i.m. (EC, E.C.P.®). The day of estrus was considered D0. Daily ultrasound (trans-rectal linear transducer of 7.5 MHz, Mindray DP 2200 vet, Mindray, China) was done between D-3 and D0 to measure the diameter of the dominant follicle (ØDF) and between D+3 and D+26 to measure CL volume. The serum concentration of P4 was determined between D+3 and D+17 and the complete luteolysis was considered when P4 <0.10 ng/mL. The research was performed in three experimental periods when each animal was submitted to all treatments (n = 54). However, only single ovulation animals were kept in the study (n = 25). The results were expressed as mean ± SEM and data was analyzed using GLIMMIX (general data) and MIXED (repeated measures) procedures of SAS 9.3 software. The significance level was 5%. The results of groups Control (n = 8), pre-eCG (n = 6) and post-eCG (n = 11) were, respectively: ØDF between D-3 and D0 - 10.79 ± 0.36, 10.57 ± 0.61 and 11.16 ± 0.33 mm (P treat = 0.83, P time <0.01, P treat*time = 0.55); CL volume between D+3 and D+17 – 5,926.0 ± 226.0, 5,968.4 ± 287.7 and 6,561.8 ± 229.6 mm3 (P treat = 0.52, P time <0.01, P treat*time = 0.04), with lower CL to pre-eCG on D+9 and lower CL to Control on D+13; serum P4 - 1.89 ± 0.12, 2.21 ± 0.15 and 2.27 ± 0.12 ng/mL (P treat = 0.40, P time < 0.01, P treat * time = 0.96), with lower P4 to Control group on D+14; luteolysis rate until D+26 - 87.5 (7/8), 83.3 (5/6) and 81.8% (9/11) (P = 0.84); interval ovulation-luteolysis - 21.1 ± 0.8, 20.8 ± 0.5 and 19.3 ± 0.6 days (P = 0.16); and interval between two ovulations - 22.8 ± 1.0, 22.8 ± 0.5 and 22.0 ± 0.5 (P = 0.68). Despite the similarity in the interval ovulation-luteolysis, it was observed that luteolysis at D+20 considering the respective treatments was 12.5 (1/8), 0.0 (0/6) and 54.5% (6/11). It was concluded that treatment with 400IU eCG after ovulation increases the volume of CL, and the same treatment, before or after ovulation, is able to increase the circulating P4 on day 14 after estrus. However, the effect of treatments on the longevity of the CL needs to be better clarified. Acknowledgements: FAPESP (2014/00739-9 and 2015/02551-0).

A234 Embryology, Developmental Biology and Physiology of Reproduction

Effects of different oocyte activation methods on the development of bovine embryos produced by parthenogenesis and intracytoplasmic sperm injection

C.F. Martins[1], H.C. Bessler[1], M.A.N. Dode[2], C.G. da Silva[3]

[1]EMBRAPA Cerrados, Brasilia; [2]EMBRAPA Recursos Genéticos e Biotecnologia, Brasilia; [3]EMBRAPA Cerrados/Universidade de Brasilia, Brasilia.

Keywords: activation, bovine, ICSI.

In the bovine, injection of spermatozoa must be accompanied by artificial methods of oocyte activation in order to achieve normal fertilization events. In this context, the objective of this study was to evaluate the effects of different oocyte activation treatments in the development bovine embryos produced by parthenogenesis and intracytoplasmic sperm injection (ICSI). Five activation treatments (T1: 7% ethanol; T2: 7% ethanol in association with 5 µM ionomycin; T3: 7% ethanol in association with 5 µM ionomycin and 6-(Dimethylamino) purine (6DMAP); T4: Two incubation in 7% ethanol with interval of 3h between the first and second activation; T5:7% ethanol in association with 5 µM ionomycin, culture in SOF for 3 h and repeat 7% ethanol incubation were used to evaluate production of parthenogenetic embryos. The best treatments during the first experiment were used in the ICSI study. For ICSI procedure, spermatozoa were pre-stimulated by incubation with groups of oocytes for 1.5 h and then were used for microinjection. One way Anova and Tukey test were used to compare the oocyte activation effects. The cleavage and parthenogenetic blastocyst rate using activation with 7% ethanol in association with 5µM ionomycin and 6DMAP (T3), and two incubations in 7% ethanol (T4) were significantly ($P < 0.05$) higher than others treatments. The T3 treatment produced significantly ($P < 0.05$) more embryos than T4 (31.34% vs 7.69%, respectively for T3 and T4). When these treatments were used for the activation of ICSI embryos, it was observed a similar blastocyst rate of 31.81% and 27.27% (for T3 and T4, respectively). We hypothesized that double incubation in 7% ethanol (T4) produced more ICSI embryos than parthenogenetic embryos ($P < 0.05$) because there was participation of spermatozoa, aiding in the activation process. : In conclusion, the use of 7% ethanol in association with 5 µM ionomycin and 6DMAP or double incubation in 7% ethanol are two good options for the activation of bovine oocytes during ICSI.

A235 Embryology, Developmental Biology and Physiology of Reproduction

Epidemiologic survey of Leptospira borgpetersenii hardjo bovis in Santa Catarina and Rio Grande do Sul between May and December 2015

B.S. Lima[1], M.R.T. Badke[2], G. Pastre[1], M.C.A. Teixeira[1]

[1]Virbac do Brasil, Sao Paulo; [2]Universidade Federal de Santa Maria, Santa Maria.

Keywords: Leptospirosis, hardjo bovis, reproduction.

The reproductive efficiency can be influenced by several factors, among these, infectious diseases are potentially able to affect reproduction in cattle. The leptospirosis are considered one of the major diseases that can affect the reproductive rates of cows causing, abortions, infertility, stillbirth calves and placental retention. The challenge for the control of leptospirosis is the identification of the serovars that are causing the outbreaks in each farm. Most of leptospirosis diagnostic laboratories in Brazil does not have the serovar hardjo bovis in routine, difficulting the publications of data of incidence and prevalence of Leptospira borgpetersenii hardjo bovis in the Brazilian cattle herd. The Leptospira borgpetersenii hardjo bovis affect reproduction, causing repetitions of estrus, embryos mortality and abortion. The aim of this work was the accomplishment of a research of the prevalence of Leptospira borgpetersenii hardjo bovis in bovine serum from farms with a history of reproductive problems in the states of Santa Catarina and Rio Grande do Sul. Analyses were performed with bovine serum samples received in Leptospirosis Diagnostic Laboratory of the Federal University of Santa Maria - UFSM, Santa Maria / RS, between May and December of 2015. In all, 1068 samples were analyzed. Samples came from 30 cities of Santa Catarina (n = 638) and 29 cities of Rio Grande do Sul (n = 430). All samples were tested by microscopic agglutination test (SAM) for detection of leptospiral agglutinins. The results obtained were expressed as a percentage, and revealed that among the 1068 samples analyzed 45% (483) were positive. In Santa Catarina, 234 (37%) of the 638 samples analyzed were positive, and in Rio Grande do Sul 249 (58%) of the 430 analyzed samples were positive. For these reasons, there is a high incidence of Leptospira borgpetersenii hardjo bovis in the studied area, suggesting the enzootic presence in the region. The high prevalence of Leptospira borgpetersenii hardjo bovis in the analyzed herds shows the importance of an integrated strategic program to control it in order to prevent reproductive problems and ensure the success of reproductive programs. Thus, the correct vaccination of animals with vaccines containing the hardjo bovis serovar in its formulation is the best tool for the control of Leptospira borgpetersenni hardjo bovis in cattle, since it reduces the occurrence of infection, reducing cases of abortion, stillbirths and other problems that might decrease the rates of reproductive programs.

A236 Embryology, Developmental Biology and Physiology of Reproduction

Gestational diagnosis and monitoring of fetal development in bitch by ultrasound

H.F.L. Ribeiro, E.B. Araujo, A.S. Ramos, S.T. Rolim Filho, T.R.L. Mesquita, V.C. Mileo, R.C. Conde

UFRA, Belem.

Keywords: bitch, ultrasound, pregnancy.

The aim of this study was to sets with precision the fetal structures that form during pregnancy in a dog submitted to artificial insemination (IA) through gestational ultrasound. The experiment was conducted from the 19th day after IA, once a week, through the ultrasound unit GE Logiq P3 Expert (6.0-13.0 MHz). In the first examination the presence of a gestational vesicle measuring 1,3 x 0,9 cm (longitudinal x cross) in the left uterine horn was observed, with fetal development features suggesting a gestation of about 15 days. In the 25th day of pregnancy, we observed a gestational vesicle measuring 4,8 x 2,3 cm, heartbeat of 234 bpm and fetal measures equivalent to 0,5 cm of biparietal diameter; 0,5 cm chest diameter; 0,6 cm abdominal diameter; 1,7 cm embryonic length, and a slight image of the articulate bone system (jaws, jaw and spine). The ultrasound examination performed with 36 days of pregnancy found articulated skeletal system with little acoustic shadow, 225 bpm heart rate, fetal measures of approximate 1,2 cm of biparietal diameter; 1,2 cm chest diameter; 1,2 cm abdominal diameter; 4,5 cm embryonic length; 7,9 x 2,7 cm diameter of the gestational vesicle, and placenta with anatomical dimensions (0,6 cm thick), defined contours, echogenicity and normal echo texture. About 46 days of pregnancy, fetus was noted with biparietal diameter 1,9 cm; 1,9 cm diameter injury; 2,3 cm chest diameter; 2,7 cm abdominal diameter; renal length of 1,2 cm; placental 0,9 cm thick; femoral length of 1,1 cm; humeral length of 1,1 cm; diameter of 4.0 cm gestational vesicle; in addition to viewing, and heart rate with ratio of about 237 bpm. To 52 days of pregnancy, the placenta with dimensions, contours, echogenicity and normal eco texture, normal amniotic fluid for the gestational phase, such joint bone seen with little acoustic shadow, fetus measuring biparietal diameter 2,0 cm; 1,7 cm in diameter injury; 3,3 cm chest diameter; 3,6 cm abdominal diameter. Noticed-if kidney lengths, femoral and humeral of 1,7 cm, 1,6 cm and 1,7 cm, respectively, and 257 bpm heart rate. With 60 days of pregnancy, the placenta was noted with normal standards measuring 0,4 cm thick, articulate bone system developed, producing posterior acoustic shadow, fetus measuring biparietal diameter 2,8 cm; 2,5 cm diameter injury; 4,0 cm chest diameter; 4,4 cm abdominal diameter, as well as renal length of 2,1 cm; femoral length of 2,2 cm; humeral length of 2,6 cm; diameter of 5,0 cm gestational vesicle, and heartbeat with frequency of approximately 217 to 228 bpm. The ultrasound examination demonstrated effectiveness for gestational and diagnostic monitoring of fetal development.

A237 Embryology, Developmental Biology and Physiology of Reproduction

Endometritis treatment with intrauterine infusion of glucose 50% in dairy cows

F.S. Rosa[1], R.M. Bonotto[1], T.C. Mendes[1], M.T. Bassani[1], G. Dalberto[1], S. Vargas[1], A.P. da Silva[1], A.C. Nino[2], J. Primon[2]

[1]FAI-Faculdades de Itapiranga, Itapiranga; [2]Médico Veterinário Autônomo, Seberi.

Keywords: uterus, PMN, antimicrobials.

The reproductive efficiency can be influenced by several factors, among these, infectious diseases are potentially able to affect reproduction in cattle. The leptospirosis are considered one of the major diseases that can affect the reproductive rates of cows causing, abortions, infertility, stillbirth calves and placental retention. The challenge for the control of leptospirosis is the identification of the serovars that are causing the outbreaks in each farm. Most of leptospirosis diagnostic laboratories in Brazil does not have the serovar hardjo bovis in routine, difficulting the publications of data of incidence and prevalence ofLeptospira borgpetersenii hardjo bovis in the Brazilian cattle herd. The Leptospira borgpetersenii hardjo bovis affect reproduction, causing repetitions of estrus, embryos mortality and abortion. The aim of this work was the accomplishment of a research of the prevalence of Leptospira borgpetersenii hardjo bovis in bovine serum from farms with a history of reproductive problems in the states of Santa Catarina and Rio Grande do Sul. Analyses were performed with bovine serum samples received in Leptospirosis Diagnostic Laboratory of the Federal University of Santa Maria - UFSM, Santa Maria / RS, between May and December of 2015. In all, 1068 samples were analyzed. Samples came from 30 cities of Santa Catarina (n = 638) and 29 cities of Rio Grande do Sul (n = 430). All samples were tested by microscopic agglutination test (SAM) for detection of leptospiral agglutinins. The results obtained were expressed as a percentage, and revealed that among the 1068 samples analyzed 45% (483) were positive. In Santa Catarina, 234 (37%) of the 638 samples analyzed were positive, and in Rio Grande do Sul 249 (58%) of the 430 analyzed samples were positive. For these reasons, there is a high incidence of Leptospira borgpetersenii hardjo bovis in the studied area, suggesting the enzootic presence in the region. The high prevalence of Leptospira borgpetersenii hardjo bovis in the analyzed herds shows the importance of an integrated strategic program to control it in order to prevent reproductive problems and ensure the success of reproductive programs. Thus, the correct vaccination of animals with vaccines containing the hardjo bovis serovar in its formulation is the best tool for the control of Leptospira borgpetersenni hardjo bovis in cattle, since it reduces the occurrence of infection, reducing cases of abortion, stillbirths and other problems that might decrease the rates of reproductive programs.

A238 Embryology, Developmental Biology and Physiology of Reproduction

Green and white teas consumption in the cell proliferation of endometrium of rats

I.C. Giometti[1], F.A.G. Santos[2], S.A. Freire[1], R. Sena e Silva[1], P.C. Papa[2], C. Castilho[1], A.P.M.M. Cardoso[1], C.B. Laposy[1], R.M.B. Nogueira[1], É.S.C. Kikuchi[1]

[1]UNOESTE, Presidente Prudente; [2]USP, São Paulo.

Keywords: Camellia sinensis, histology, reproduction.

The catechins presents in the green and white teas (originated from Camellia sinensis plant) are associated at reducing of cell proliferation in different kinds of cancers, with prevention of cardiovascular diseases and osteoporosis and they possess antioxidative and antibacterial activity. Despite the benefits of consumption of these teas, their catechins inhibit cell proliferation and steroidogenesis in swine granulosa cells in vitro cultivated. So it is also important to get an insight on the possible reproductive-related consequences of the use of green and white teas. Thus, the aim of this work was to verify the green and white teas influence in the cell proliferation in the endometrium of rats. For this purpose, 90 rats were divided into three groups, control group (CT, which is drinking only water ad libitum); GT group (which is drinking only commercial green tea, Amor à Vida®, Amor à Vida Produtos Naturais, Brazil); and WT group (which is drinking only white, Chá & Cia®, Brazil). The teas were prepared daily at 2.5% for infusion and offered to rats in an experimental situation as realized by Yang et al. (Eur J Cancer Prev, 12:391–395, 2003) e Niwattisaiwong et al. (Drug Metabol Drug Interact., 20:43-56, 2004). The rats were kept in plastic boxes (5 animals per box), under normal conditions, 12 hour light: 12 hour dark lighting schedule, with free access to beverage and food. This study was approved by ethics committee to be realized (numbers protocols 325 and 336). The experiment had last for three months, and in the end of each month, 10 animals of each group were superovulated with 150UI/Kg of eCG (Folligon®, Intervet Schering-Plough, Brazil) and 150UI/Kg of hCG (Vetecor®, Hertape Calier, Brazil) and sacrificed. The uteri were collected in Davidson solution and they were histologically analyzed by AgNor (Argyrophilic Nuclear Organizer Regions) method to detect endometrial cell proliferation. Photos of the uteri slides were taken at 1000x magnification and the areas of nuclear organizer regions (Nor) and nuclear areas were defined using software MOTIC Imageplus 2.0® (MOTIC, China), in 10 different camps of each slide, which 10 nucleus of were measured for camp, totalizing 100 cells for animal. The statistical analysis realized was ANOVA with Tukey, the differences were considered when $P < 0.05$. The endometrium of rats treated with teas presented minor ratio between nucleus and Nor compared to control in all moments analyzed ($P < 0,0001$): 30 days (CT = 4,81 ± 0,26a, GT = 3,38 ± 0,22b, WT = 1,44 ± 0,04c); 60 days (CT = 6,10 ± 0,42a, GT = 1,79 ± 0,03b, WT = 2,27 ± 0,40b); 90 days (CT = 6,45 ± 0,62a, GT = 1,92 ± 0,03b, WT = 3,25 ± 0,31b). The conclusion is the green and white teas consumption decreases the endometrial proliferation in rats. Financial support was provided by FAPESP (2010/20583-2).

A239 Embryology, Developmental Biology and Physiology of Reproduction

Expression of genes involved in oocyte competence is higher in cumulus cells of Nelore cows with low compared to high number of antral follicle

C.O. Rosa[1], L.S.R. Marinho[1], P.R. Antunes da Rosa[2], M.P. de Cesaro[2], P.A. Lunardelli[1], K.C. Silva-Santos[1], V. Bordignon[3], M.M. Seneda[1]

[1]UEL, Londrina; [2]UFSM, Santa Maria; [3]Mcgill, Sainte-Anne-de-Bellevue.

Keywords: antral follicles, competence oocyte, cumulus cells.

The objective of this study was to investigate if the number of antral follicles (AF) is correlated with oocyte competence. Ovaries (n = 712) from Nelore cows (n = 356) were obtained at the slaughterhouse and divided in groups according to the number of antral follicles. The mean quantity of follicles was 61.14 ± 30.43 per cow. The experimental groups were defined as: females with ≤ 31 AF (mean number – SD); 46-76 AF (mean number ± ½ SD); and ≥ 92 AF (mean number + SD). Cumulus-oocyte complexes (COCs) were collected from each group, matured, fertilized and cultured in vitro to assess developmental competence to the blastocyst stage. COCs (n = 180) were used to quantify mRNA expression by qRT-PCR in 3 replicates. Total RNA was extracted from granulosa and cumulus cells using Trizol. RNA purity and quantity were determined using a Nano-Drop spectrophotometer, and then 200 ng total RNA per sample was reverse transcribed using the iScript cDNA synthesis kit (BioRad, ON, Canada). Quantitative Real time PCR was performed using a CFX384TM Real-Time System (BioRad) and iQ SYBR Green Supermix (BioRad). mRNA abundance of tested genes was normalized to the average of the internal control genes RP18S and Cyclophilin. Data were submitted to ANOVA and the averages compared by Tukey's HSD test. Embryo development data were analyzed by logistic regression and differences were considered statistically significant if $P < 0.05$. There was no difference between groups for cleavage and development to the blastocyst stage. In cumulus cells, samples from cows with ≤ 31 AF had higher mRNA abundance of genes involved in meiosis resumption (NPR-2 and NPR-3), estradiol biosynthesis (CYP19A1), cumulus cells expansion (FGF10), and transcription factors that regulate oocyte maturation and cell proliferation (STAT3 and FOXO3a). There was no difference in mRNA abundance of STAR (involved in steroidogenesis), NPR-1, PGRMC1, PGR (involved in meiosis resumption), LIFRα (transcription factor that regulate oocyte maturation and cell proliferation), FGF2Rb, FSHR, BMPR2, AMH, AMHR2 and TGFßR1 (involved in cumulus cells expansion) in cumulus cells. In granulosa cells, NPR-1 mRNA levels (involved in meiosis resumption) were higher in the group with ≥ 92 AF. Findings from this study revealed molecular differences related to meiosis resumption, synthesis of estradiol, cumulus cells expansion, oocyte maturation and cell proliferation in cumulus cells of Nelore cows of low vs. high number of AF.

A240 Embryology, Developmental Biology and Physiology of Reproduction

Follicular steroids cooperate with NPPC to delay nuclear maturation and to increase oocyte-cumulus communication in cattle

A.C.S. Soares[1], V. Lodde[2], L. Sanches[1], R.G. Barros[1], P.F. Lima[1], C.A. Price[3], A.M. Luciano[2], J. Buratini[1]

[1]UNESP - Campus Botucatu, Botucatu; [2]Università Degli Studi Di Milano, Milão; [3]Universidade de Montreal, Saint Hyacinthe.

Keywords: oocyte maturation, gap junctions, germinal vesicle.

Oocyte maturation is precociously induced when the cumulus oocyte complex (COC) is removed from the follicle for IVM/IVF, which accounts for the limited efficiency of IVM. Natriuretic peptide type C (NPPC) delays GVBD and increases oocyte-cumulus communication. In rodents, estradiol appears to cooperate with NPPC by increasing the expression of its receptor NPR2. In this study we first tested the hypothesis that follicular steroids (estradiol, progesterone and androstenedione) at approximately physiological levels cooperate with NPPC to delay GVBD (Experiments 1) and to maintain oocyte-cumulus communication (Experiment 2). Then, we assessed the effects of follicular steroids, NPPC and the combination of both in a pre-IVM culture on embryo production (Experiment 3). Experiments 1 and 3 were performed at UNESP-Botucatu, with ovaries from Bos indicus (predominantly Nellore), and experiment 2 was conducted at the University of Milan with ovaries from Bos taurus (Holstein). COC were aspirated from 3-8mm follicles from abattoir ovaries and cultured in groups of 20. Base medium was TCM199 supplemented with BSA (0,4%), amicacin (75µg/mL) and pyruvate (22µg/mL). In Experiments 1 and 2, COCs were cultured for 9 hours in base medium only (B) or base medium supplemented with NPPC (NPPC), base medium supplemented with follicular steroids (FE) or follicular steroids plus NPPC (NPPC+FE; concentrations were within ranges described in the patent PCT 201690005). At the end of culture, the proportion of oocytes at the germinal vesicle (GV) stage (Experiments 1), and the proportion of COCs with opened gap junctions communications (GJC) were assessed (Experiment 2). To identify GV stage, oocytes were denuded and stained with Hoechst 33342 and GJC were assessed by examining the transfer of the dye injected (Lucifer Yellow) from the oocyte to cumulus cells. Effects of treatments were analyzed by ANOVA followed by Tukey's test. Experiment 3 tested the effects of the treatments above as pre-IVM culture step on embryo production. For IVM, COCs were cultured in 400µl of base medium supplemented with 100ng/ml amphiregulin (AREG) at 38.5ºC and 5% CO2 in humidified air for 24h. In Experiment 1, FE+NPPC maintained oocyte GV arrested as at the time of collection (96.1 vs. 97.5, $P > 0.05$). Moreover, NPPC increased the percentage of COC with open GJC in B+NPPC group compared to B group (47.2 vs. 24.2, $P < 0.05$) and to a level similar to the control before culture in FE+NPPC group (60.3 vs. 83.1, $P > 0.05$). Blastocyst production and the percentage of hatched and expanded blastocysts did not differ between treatments. In conclusion, the data suggest that follicular steroids enhance the ability of NPPC to maintain meiotic arrest and to increase oocyte-cumulus communication. Further studies are in progress to ascertain the effect of delayed nuclear maturation with FE+NPPC on embryo development after transfer.

A241 Embryology, Developmental Biology and Physiology of Reproduction

Effects of the inhibitor of actin polymerization, "Latrunculin A" during bovine oocyte parthenogenetic activation

G. Vans Landschoot[1], S. Ferraris[1], D. Salamone[2]

[1]Centro de Investigación Y Desarrollo En Medicina Experimental (CIDME), Universidad Maimónides., Buenos Aires;
[2]Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires.

Keywords: Latrunculin A, parthenogenetic activation, bovine.

Somatic cells nuclear transfer (SCNT) protocols require artificial activation to mimic fertilization and induce embryo development. However, the chemical activation treatments available can result in chromosomal aberrations (Kim et al., Biol. Reprod., 54, 1397-404, 1996). For this reason, it is necessary to develop improved activation protocols. Recent improvements in NT protocols, such as the use of actin polymerization inhibitor, latrunculin A (LatA), rather than cytochalasin B (CB), contributed to enhance the cloning efficiency (Mallol et al., PLoS ONE, 10, doi:10.1371/journal.pone.0120033, 2015). The aim of this study was to define the optimal LatA concentration to induce diploid activation in parthenogenetic bovine embryos, in combination with Roscovitine (Rosco). For that, COCs were collected from cow ovaries obtained from slaughterhouses and in vitro matured in TCM 199 supplemented with 5% FCS, 10 UI mL-1 penicillin, 10 µg mL-1 FSH, 100 µM cysteamine and 0.3 mM sodium pyruvate, at 39°C under 6.5% CO2 in air for 20 h. After removal of cumulus cells, metaphase II (MII) oocytes were treated with 5 µM ionomycin (Io) for 4 min and randomly assigned to the following activation groups: a) LatA 1µM/Rosco (5 mM Roscovitine for 5 h); b) LatA 5 µM/Rosco; c) LatA 10 µM/Rosco; d) LatA 20 µM/Rosco; e) Control group: 2 mM 6-Dimethylaminopurine (DMAP) for 3 h. Activated oocytes were cultured in SOF medium and rates of blastocysts were evaluated on day 7 of development. Results: Blastocyst rates were lower in the groups treated with Io followed by: LatA 1 µM/Rosco (n = 108, 18.52%), LatA 5 µM/Rosco (n = 90, 26.67%), LatA 10 µM/Rosco (n = 90, 24.44%) and LatA 20 µM/Rosco (n = 65, 16.92 %) than in DMAP treated group (n = 90, 31.58 %). Results showed that DMAP rates were significantly higher than LatA 1 µM/Rosco and LatA 20 µM/Rosco (31.58, 18.52 and 16.92 %, respectively) (Fisher's test, $P < 0.05$). Finally, Io followed by LatA 5 µM/Rosco and LatA 10 µM/Rosco, resulted in developmental rates similar to Io-DMAP treatment ($P > 0.05$), but the ploidy level of the embryo remaim to be determine. As LatA demonstrated less chromosomal abnormalities (Terashita et al., Plos ONE, 8:e78380. doi: 10.1371), the results are encouraging for use in SCNT, as it might increase the normal embryos.

A242 Embryology, Developmental Biology and Physiology of Reproduction

The oocyte regulates GDF9 and BMP15 expression in bovine cumulus cells

P.F. Lima[1], R.G. Barros[1], J.N. Sakoda[1], A.C.S. Soares[1], C.A. Price[2], J. Buratini[1]

[1]UNESP, Botucatu; [2]Universidade de Montreal, Saint Hyacinthe.

Keywords: cumulus cells, BMP15, AREG.

Unlike in the mouse, the presence of the oocyte is not required for expansion of the cumulus-oocyte complex (COC) in cattle. However, we have previously demonstrated that oocyte secreted factors, although not absolutely required, do enhance cumulus expansion in cattle. Major oocyte secreted factors regulating COC maturation are members of the TGFβ family, BMP15 and GDF9. Previous data suggest that BMP15 and GDF9 are expressed in bovine cumulus cells. Thus, the existence of an intra-cumulus loop mediated by TGFβ signalling in cattle constitutes an interesting hypothesis. In this study, we tested the hypothesis that BMP15 and GDF9 are expressed in cumulus cells during IVM in cattle and that mRNA abundance is regulated by the oocyte.COCs were aspirated from follicles 3-8 mm in diameter and COCs (grade 1 or 2) were divided in three groups: intact COCs (COC), oocytectomized COCs (OOX), and OOX plus denuded oocytes (DO). Oocytectomy was performed by aspiration of the ooplasm with a micromanipulator. COCs were matured in groups of 20 for 4 or 22 hours (n = 4/time). IVM was performed in TCM199 with Earl's salts supplemented with either FSH (1μg/mL) or AREG (100 μg/mL), 0.4% BSA, 22 μg/Ml sodium pyruvate, 75μg/mL of amikacin at 38,5°C and 5.5%CO2 in humid atmosphere. Abundance of mRNA encoding BMP15 and GDF9 in cumulus cells was assessed by real-time RT-PCR using Power SybrGreen (LifeTech®) and CYC-A as the housekeeping gene. Effects of treatments were analysed by ANOVA and groups were compared with the Tukey test.Expression of BMP15 and GDF9 mRNA was detected in bovine cumulus cells. PCR cycle numbers were on average 29 and 28 for BMP15 and GDF9, respectively, at 4 hours of culture, and 29 and 31, at 22 hours of culture. Oocytectomy significantly reduced BMP15 and GDF9 mRNA expression in COCs cultured with AREG for 4 or 22 hours, but not with FSH. Co-culture of OOX with DO restored BMP15 and GDF9 expression . In conclusion, these data suggest that BMP15 and GDF9 mRNA expression in bovine cumulus cells is regulated by the oocyte. This effect is abolished by supra-physiological levesl of FSH used in IVM in cattle.

A243 Embryology, Developmental Biology and Physiology of Reproduction

Estradiol changes the immunohistochemical profile of the endometrial proteins gamma PKC, AKR1B1 and alpha estradiol receptor in cattle

B.P. Mello[1], A.M. Gonella-Diaza[1], G. Pugliesi[1], S.C. Scolari[2], M. Binelli[3], C.M.B. Membrive[1]

[1]USP, Pirassununga; [2]Faef, Garça; [3]UNESP, Dracena.

Keywords: endometrium, PGF2α.

In cattle estradiol (E_2) has an important role in the endometrial PGF2α release associated with luteolysis, however, the molecular mechanisms involved in such process are poorly understood . The PGF2α synthesis is the result of a series of intracellular events that include the participation of kinase C gamma protein (PKCγ), aldo-keto reductase family 1 member B1 (AKR1B1) and estradiol receptor α (ERα) which are present in the endometrial cells. The main hypothesis was that females treated with E2 have a modification of PKCγ, AKR1B1 and Erα endometrial protein concentration. The objective of this study was to investigate using immunohistochemistry the PKCγ, AKR1B1 and ERα proteins in endometrial tissue in Nelore cows treated or not with 3 mg of 17β - estradiol intravenously on the 15th day of the estrous cycle 0, 4 and 7 hours after injection. Nelore (n = 52), pluriparous, cyclic and non-lactating cows received 2 mg of estradiol benzoate (Sincrodiol Ourofino®, Cravinhos, Brazil) and an intravaginal progesterone device (1g; Sincrogest; Ourofino®, Cravinhos, Brazil) during 8 days. The cows received 0.5 mg of sodium cloprostenol (Sincrocio; Ourofino®, Cravinhos, Brazil) via IM, 48 hours before the withdrawal of the device and a second application the day of device removal. On day 15 of the estrous cycle (D0; estrus) the following treatments were administered: placebo (P; 5 ml of ethanol 50%; IV), estradiol (E; 5mL of 50% ethanol containing 3 mg of 17β estradiol; IV) or control (not treated). Time 0 was the moment of the treatment application. Cows were subjected to a transcervical endometrial biopsy, and according to the time of biopsy were divided into the following groups: time 0 in the control group (C; n = 10), time 4 hours (E4, n = 11 and P4, n = 10), and 7 hours (E7, n = 10 and P7; n = 11). The procedures performed are in accordance with the principles of the Ethics in AnimalResearch Committee of UNESP Dracena/SP (CEUA Protocol 08/2014). The tissue obtained by biopsy was placed in 4% buffered-formalin- for 24 hours and then stored in 70% alcohol until paraffin embedding. Endometrial sections were evaluated by immunohistochemistry and immunostaining was evaluated in the luminal epithelium (LE), glandular epithelium (GE) and stroma (S). The statistical differences were determined by t test and considered when $P < 0.05$. The results of PKCγ protein showed higher immunostaining in the LE of E4 and E7 groups compared to P4 and P7 ($P < 0.05$) and increased labeling in GE of E7 compared to P7 ($P < 0,05$). The AKR1B1 protein showed higher immunostaining in the LE of E4 and E7 groups compared to P4 and P7 ($P < 0,05$) and higher immunostaining in GE of E4 compared to P4 ($P < 0,05$). The ERα shows an higher immunostaining in the GE of P4 and P7 groups compared to E4 and E7 ($P < 0.05$) and higher immunostaining in LE of P4 when compared to E4 group ($P < 0.05$). It is concluded that E_2 increases immunostaining of the PKCγ and AKR1B1 proteins and reduce of the Erα protein in endometrial tissue, thereby modifying the concentration of these endometrial receptors.

A244 Embryology, Developmental Biology and Physiology of Reproduction

Evidence that fibroblast growth factor 2 is involved in mechanisms by which the oocyte regulates its own nuclear maturation

R.G. Barros[1], D.J. Sartor[1], P.F. Lima[1], A.C.S. Soares[1], L. Sanches[1], C.A. Price[2], J. Buratini[1]

[1]UNESP - Botucatu, Botucatu; [2]Universidade de Montreal, Saint Hyacinthe.

Keywords: cumulus cells, bovine, maturation.

Fibroblast growth factor 2 (FGF2) expression is upregulated by the LH ovulatory peak in bovine cumulus cells (CC). In bovine granulosa cells, FGF2 activates ERK1-2, a pathway known to stimulate oocyte nuclear maturation and cumulus expansion. We have previously observed that FGF2 increases CC expansion during in vitro maturation of bovine cumulus-oocyte complexes (COC). The aims of this study were to assess the effects of FGF2 on germinal vesicle breakdown and investigate if FGF2 expression in cumulus cells is regulated by the oocyte. Effects of FGF2 on the expression of genes regulating oocyte nuclear maturation were also assessed. Groups of 20 COCs (grades 1 and 2) from 3-8mm follicles of abattoir ovaries were cultured in base medium TCM 199 with Earle's salts supplemented with 0.4% BSA, 1µl/mL FSH, 22µg/ml sodium pyruvate, 75µg/ml amicacin at 38,5°C in humid atmosphere. To assess oocyte nuclear maturation and CC gene expression, COC were cultured in 450µL of base medium with grading doses of FGF2 (0, 1, 10 and 100ng/mL; n = 5/time/dose) for 6 and 22 hours. After culture, CC and oocytes were mechanically separated, and oocytes were stained with Hoechst 33342 to assess germinal vesicle breakdown (GVBD) at 6 hours, and metaphase stage (MI or MII) at 22 hours. Abundance of mRNA encoding AREG, EREG, NPPC and NPR2 was assessed by real time qPCR normalized to CYC-A in CC from 6 hours culture. To assess the influence of the oocyte on FGF2 expression, intact COCs (COC), oocectomized COCs (OOX) and OOX+denuded oocytes (DO) were cultured in the medium above in 100 µL for 4 and 22 hours. Effects of treatments were tested by ANOVA or Kruskal-Wallis and means were compared by the Fisher protected test. FGF2 increased the percentage of GVBD oocytes (18.89 ± 5.86a, 43.36 ± 6.68b, 33.63 ± 6.55ab, 26.88 ± 7.47a %; for 0, 1, 10 and 100ng/mL, respectively), without affecting meiosis progression at 22 hours or gene expression. Expression of FGF2 in CC was not influenced by the oocyte at 4 hours. At 22 hours, however, FGF2 mRNA abundance was lower on OOX compared with intact COCs. The addition of DO to OOX did not entirely restore FGF2 expression (4.41 ± 0.74a, 1.82 ± 0.12b, 3.19 ± 0.55ab for COC, OOX and OOX+OD, respectively), suggesting that the influence of the oocyte may be only partially regulated by oocyte secreted factors, and also involve direct cell communication. In conclusion, the present data suggest that FGF2 is involved in mechanisms leading to GVBD. As FGF2 expression is regulated by the oocyte, it may represent a mechanism by which the oocyte regulates its on maturation.

A245 Embryology, Developmental Biology and Physiology of Reproduction

Morphological analysis, cytological, and oocyte quality of reproductive tract of bovine females submitted to slaughter

L.M. Sena, Í.C. de Almeida, N.C. Lazaroni e Merchid, J.D. Santos, T. de Castro, B.C.S. Albuquerque, M.A.P.L. Chieppe, J.O. Carvalho, C.B. Martins

Universidade Federal do Espírito Santo, Alegre.

Keywords: cumulus cells, uterine infection, neutrophils.

The disorders that cause infertility and subfertility in cows can be either infectious or not, or originate from errors in breeding and nutrition. Uterine infections are among the main causes of infertility, negatively influencing ovarian activity and reproductive performance of animals. Thus, the purpose of this study was to characterize the main reproductive disorders through macroscopic and cytologic evaluations of the uterus of cows, focusing on the disorders that cause a decrease in reproductive efficiency, and to correlate the different degrees of uterine infection with oocyte quality. This research was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Espírito Santo, under number 76/2015. 98 reproductive tracts were obtained from slaughterhouses. In relation to macroscopic aspects, changes related to morphology, consistency and color were evaluated. Inflammation was classified according to the aspect of the uterine secretion as: modera clinical infection (grade II): presence of cloudy or catarrhal mucus; severe clinical infection (grade III): presence of purulent mucus (Ferreira et al, Brazilian Agricultural Research 22: 339-344, 1987). The diagnosis of subclinical uterine infection (grade I) was conducted by endometrial cytology obtained by endometrial scraping. Subclinical infection was characterized by the presence of more than 3% neutrophils, from the 200 cells counted (Salasel et al, Theriogenology, 74: 271-1278, 2010). The oocyte quality of each ovary/animal was analyzed according to the number of cumulus cell layers and cytoplasm aspect, and was classified as: grade I: excellent quality, grade II: intermediate, and grade III: poor. The data was analyzed using Descriptive Statistical Analysis, Spearman Correlation test, and Post-compared with Student's t test, with 5% significance level. In regards to ovarian changes, 1.02% of ovarian cysts, ovarian agenesis and ooforites were observed. 4.08% of the animals had uterine wall thickening. 1.02% of the animals had an increase in length of the uterine horns, uterine fibrosis, and the presence of clots. There were 12.24% of uterine clinical infections cases. 6.12% of the animals with positive endometrial cytology were diagnosed with subclinical uterine infection. In the analysis using the Spearman Correlation test, there was a negative correlation ($r = -0.14$), with no significance between uterine infection and rates of recovered oocytes grade I and II.Clinical and subclinical uterine infections were the most frequent changes are possible causes of decreased fertility. There wasn't significant correlation between the degree of uterine infection and oocyte quality of animals.

A246 Embryology, Developmental Biology and Physiology of Reproduction

Reproductive seasonality in Saanen goats managed in southeast Brazil

M.F.A. Balaro[1], A.S. Santos[1], S.G.V. Mello[1], L.M. Cavalcanti[1], L.H.S. Gonçalves[1], L.F.G.M. Moura[1], J.M.G. Souza Fabjan[1], J.F. Fonseca[2], F.Z. Brandao[1]

[1]Universidade Federal Fluminense, Rio de Janeiro; [2]EMBRAPA Caprinos e Ovinos, Sobral.

Keywords: progesterone, estrus cycle, cyclicality.

The aim of this study was to characterize the reproductive seasonality during the period of one year in Saanen goats managed in southeastern Brazil. The study was carried out in a dairy goat farm located in Niteroi, Rio de Janeiro (Latitude 22° 52 ' 30 " south). A total of 24 Saanen goats (3.2 ± 2.1 years old) were used and kept under intensive management system. Every three months, representing each season of the year, was conducted the goats weighing in a proper balance and the evaluation of the body condition score (BCS) (Detweiler, G., Annual Goat Field Day, v.23, p.127-133, 2008). Every 14 days, the blood was collected through jugular venipuncture in vacuum tube (Vacutainer®, BD, New Jersey, USA) without anticoagulant, to obtain serum. The progesterone (P4) concentration was analyzed by Radioimmunoassay technique using commercial kits (MP Biomedicals, Inc, Orangeburg, NY). For the calculation of cyclicality frequency, it was considered as non-cyclical goats, animals with values of P4 ≤ 1.0 ng/mL for two consecutive measurements. The body weight, BCS and hormonal data were submitted to Kruskal-Wallis test and Dunn ($P < 0.05$). No differences ($P > 0.05$) in the body weight and BCS among the four seasons (65.1 ± 22 kg; BCS: 3.4 ± 0.4) were found. With respect to P4 concentrations and the frequency of cyclical goats, it were obtained greater values ($P < 0.05$) from late-March until the early-August (3.3 ± 1.1 ng/mL) with a peak from May to July (3.9 ± 0.5 ng/mL). In these months, 93.8% (22/24) of the goats were cyclical, compatible with the breeding season. From the late-August to the mid-December were obtained the lowest ($P < 0.05$) P4 values (0.2 ± 0.4 ng/mL). In this period of time, only 12.5% (3/24) of goats extended the breeding season until the mid-September. In sequence, 100% (24/24) of the goats came into seasonal anestrus. The back of reproductive activity and P4 values, similar to those found in previous station, began in mid-December to early-March (3.9 ± 2.6 ng/mL) with a peak at the late-February until March (6.7 ± 2.3 ng/mL). From December to March, 38.9% (9/24) and 61.1% (15/24) of the goats showed continuous and alternating estrus cycles (transition phase), respectively. In the peak period (Feb-mar), 83.3% (20/24) of the goats were cyclical. These results endorse hormonal methods (estrus synchronization and induction of synchronized estrus) or natural (male effect, flushing or light treatment) more appropriate according to the time of year in the region. Saanen goats, managed under tropical climate in southeastern Brazil, have a breeding season set from March until mid-August with the transitional phase to the anestrus in September. It is extends to mid-December at the new one transition phase for the reproductive season in March.

A247 Embryology, Developmental Biology and Physiology of Reproduction

Reproductive parameters of dairy herds on different systems in west and southwest regions of Paraná state

T. Minela[1], F. Bracht[2], J.M.C. Mendonça[2], T.O. Cunha[2], T.S. Balestrin[1], A.P. Martini[1], G.A. Pessoa[3], J.M. Trentin[3], M.F. Fiorenza[3], M.I.B. Rubin[1]

[1]UFSM, Santa Maria; [2]B&M Consultoria Agropecuária Ltda, Cascavel; [3]UFRGS, Porto Alegre.

Keywords: dairy cattle, embryonic death, somatic cell count.

The reproductive efficiency in dairy cattle is influenced by several factors. Through many challenges, sometimes the cow just does not perform the genetic potential as expected. Data analyzed was provided by B&M Consulting Agricultural Ltda, from Sep/2015 to Apr/2016 in 15 dairy herds (n = 15, n = 746 cows), located in the western and southwestern regions of Paraná state, Brazil, on different farming systems: Compost Barn (CB = 1, n = 48 cows), Freestall (FS = 2, n = 212 cows) and Non-confined (NC = 12, n = 486 cows). In all systems, cows were inseminated after natural estrus manifestation, or submitted to synchronization of estrus and ovulation programs with progestagen (Pereira et al 2015 J. Dairy Sci 98: 947-959). All animals were examined by ultrasound (US) monthly. It was considered embryonic death (ED) ≤60 days. The effect of the Somatic Cell Count bulk tank (x103/ml milk) and milk yield (kg/cow/day) were evaluated as follows: days in milk (DIM), days open (DO) and embryonic death. The effect of high temperature (T°C) with average ≤25°C (Sep/15, Oct/15, Nov/15 and Apr/16) or ≥25°C (Dec/15, Jan/16, Feb/16 and Mar/16) was evaluated on milk production, SCC, DIM, DO and ED was evaluated. Data were analyzed by PROC GLIMMIX, PROC REG in SAS 9.3 program. The milk production (kg/cow day) in the CB system was 17.0 ± 2.3 kg/cow/day, in SF 21.9 ± 0.33 kg/cow/day and in NC 20.4 ± 1.38 kg/cow/day, being similar between the systems and did not affect reproductive parameters. The ED was higher (P = 0.03) in the FS system (62.5 ± 3.6%) compared to the CB and NC (38.5 ± 0.5 and 18 ± 0.5%, respectively). SCC in the tank was higher (P = 0.0384) in the FS system (2266 ± 987.9x10³/ml) compared to CB (817.5 ± 13.5x10³/ml) and NC (782.4 ± 114.2x10³/ml) systems. Increased SCC also raised the occurrence of embryonic death (P = 0.0004, R^2 = 0.627, ME = -0.03+ 0.00028*SCC). All systems had a negative effect of heat on milk yield (P < 0.0001), the mean of months with T ≥25°C was 19.4 ± 1.0 kg/cow/day and months with T°C ≤25°C was 21.1 ± 1.28 kg/cow/day. The DIM of herds was influenced by the weather (P = 0.0027). DIM raised (220 ± 11.1 days) during hot months while months of mild temperature this value was 199.3 ± 10.3 days. There was interaction between the farm systems and temperature (P < 0.0001). The mean DIM was higher in the FS system (241 ± 33 and 273 ± 24.5 days); intermediate in the CB (234.7 ± 5 and 272.5 ± 7 days); and smaller in the NC (189.3 ± 10.3 and 206.1 ± 9.9 days) in months average ≤25°C or ≥25°C, respectively. The temperature of ≤25°C (161 ± 14.1 days) or ≥25°C (163.5 ± 12.4 days) (P = 0.5541) had no effect on DO, however it was higher (P < 0.0001) in the FS (223.1 ± 37.1 days), followed by CB (201.63 ± 11 days) and NC (149 ± 13.04 days). Increased SCC was associated with increased embryonic death in the herds. Temperature and management practices in each system had a detrimental effect on the reproductive efficiency.

A248 Embryology, Developmental Biology and Physiology of Reproduction

Insulinogenic diet increases circulating progesterone soon after feeding, but does not change gene expression of liver enzymes that metabolize progesterone in non-lactating Holstein cows

C. Spies[1], A.M.O. Canavessi[1], M.M. Guardieiro[2], P.L.J. Monteiro Junior[1], L.H. Oliveira[1], L.F.E. Melo[1], J.N. Drum[1], J.Z. dos Anjos[1], A. dos Anjos[1], R. Sartori[1]

[1]ESALQ/USP, Piracicaba; [2]Lanagro, Campinas.

Keywords: progesterone, insulin, hepatic catabolism.

Some researchers support the idea that progesterone (P4) metabolism decreases under high circulating insulin due to lower gene expression of liver enzymes. Moreover, it was demonstrated that dry matter intake increases liver blood flow and promotes greater catabolism of steroid hormones. The objectives of this study were to evaluate circulating P4 concentrations before and after feeding maintenance diets (isocaloric and isonitrogenous) containing different carbohydrate sources (corn - more insulinogenic vs. citrus pulp - less insulinogenic) and to compare the gene expression of enzymes that metabolize P4. The experimental diets were offered for 70 d to 22 non-pregnant and non-lactating Holstein cows in a crossover design. The evaluated liver enzymes were: Cytochrome P450, family 2, subfamily C, polypeptide 19; (CYP2C19); Cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4); Aldoketoreductase, family 1, C4 member (AKR1C4); Aldoketoreductase, family 1, member D1 (AKR1D1). Cows were synchronized and superovulated as follows: d0: P4 implant + EB; d4 to d7: 2 daily doses of FSH - 300mg in total; d6: two doses of PGF2α; d8: GnRH a.m. and AI p.m.; d9: AI a.m.; d15: uterine flushing. Liver biopsy was performed 8 d after estrus, 4 h after feeding, in the end of each replicate. Liver samples were kept in RNAlater at -80°C until analysis. Blood was sampled 6 d after estrus immediately before feeding for insulin and P4 evaluation by RIA and 4 h later, for P4 assessment. Gene expression analysis (RT-qPCR) was performed using LightCycler 480 Sybr Green Master kit (Roche) according to manufacturer's instructions. Statistical analysis was performed using the PROC MIXED of SAS 9.3 for hormone concentrations. Gene expression data were analyzed using delta-delta CT and PROC GLIMMIX of SAS 9.3. Circulating insulin concentration was 15.8 ± 0.9 and 11.5 ± 0.9 μIU/mL for corn and citrus pulp, respectively ($P < 0.01$). Circulating P4 concentrations 4 h after feeding increased 17% vs. 6% in corn and citrus pulp groups, respectively ($P < 0.01$). Gene expression of enzymes that metabolize P4 was similar between treatments and the relative expression of corn vs. citrus pulp, for the enzymes studied, was respectively: CYP2C19 – 0.26 vs. 0.27 ($P = 0.87$); CYP3A4 – 0.19 vs. 0.10 ($P = 0.46$); AKR1C4 – 0.16 vs. 0.13 ($P = 0.37$) and AKR1D1 – 4.47 vs. 3.87 ($P = 0.41$). Moreover, there was no difference ($P > 0.10$) in the relative expression of the enzymes when higher vs. lower circulating insulin was compared, regardless of treatment. In conclusion, although no influence in gene expression of enzymes that metabolize P4 was found, insulinogenic diet appears to stimulate an immediate increase in P4 production.

A249 Embryology, Developmental Biology and Physiology of Reproduction

The bovine embryo modulates endometrial function 7 days after estrus *in vivo*

M. Sponchiado[1], N.S. Gomes[1], P.K. Fontes[2], M.I.S. da Silva[1], T. Martins[1], G. Pugliesi[1], M. Del Collado[1], A.A. Pastore[3], M.F.G. Nogueira[2], M. Binelli[1]

[1]Universidade de São Paulo - USP, Pirassununga; [2]UNESP, Botucatu; [3]Crv Lagoa, Sertãozinho.

Keywords: pre-implantation embryo development, endometrium, gene expression.

The maternal reproductive tract plays a critical role in the success of early embryonic development. Oviductal and uterine secretions provide a unique environment for establishment and maintenance of pregnancy. The bovine embryo produces interferon-tau (IFN-t) from as early as 6 days after AI. However, to the best of our knowledge, there is no evidence that the embryo affects endometrial function before elongation, which starts 13 days after AI. Hypothesis was that embryo affects endometrium function, including response to IFN-t, as early as 7 days after AI, in a spatial-specific manner. Specifically, endometrial responses to the embryo were expected to decrease from regions proximal to distal to the oviduct. Estrous cycles of multiparous, non-lactating Nelore cows were synchronized and animals were allocated to one of two experimental groups at estrus (D0): Control (Con; n = 8), cows were sham-inseminated and received semen diluent; or Pregnant (Preg; n = 16), cows were inseminated with semen from the same batch of a commercial bull, 12h post estrus. Cows were slaughtered on D7 and the uterine horn ipsilateral to the CL was isolated and divided in anterior, middle and posterior thirds, starting from the uterotubaric junction (UTJ). Each uterine third was washed individually with D-PBS and presence of an embryo in the flushing was confirmed in the Preg group. Subsequently, intercaruncular endometrial samples were collected from UTJ and from each uterine third in the mesometrial region. Relative abundance of transcripts for Interferon stimulated genes (ISGs; MX1, MX2, ISG15, OAS1, IFI6 and IRF6), Interferon Receptor 2 (IFNAR2), Prostaglandin E synthase (PTGES) and reference genes (GAPDH, PPIA and ACTB) were evaluated on endometrial samples by PCR using Fluidigm platform (Biomark HD). Relative abundance of transcripts on endometrium was analyzed using split-plot ANOVA (SAS v. 9.3). Size of the pre-ovulatory follicle, subsequent CL area and plasmatic P4 concentrations were similar between groups ($P > 0.1$). All embryos found (n = 10) were in the anterior third flushings. There was an interaction between group and region for abundance of ISG15 ($P < 0.02$), MX2 ($P < 0.03$), MX1 ($P < 0.07$) and PTGES ($P < 0.05$) transcripts. Abundances of ISG15, MX1, MX2 and PTGES were greater (2.0, 1.5, 1.95 and 1.4-fold, respectively) in the UTJ of Preg cows, but were similar in other regions. Abundance of IFNAR2 was affected by region ($P < 0.04$), and was greater in the UTJ than in the remaining regions. In summary, we propose that embryo-released IFN-t acts through specific endometrial receptors to stimulate transcription of ISGs and possibly other genes. Moreover, physical proximity to the embryo seems to be required for stimulating the endometrium, probably because of the limited capacity of synthesis and secretion of signals by the early embryo. This is the first time that such early endometrial response to the embryo is reported *in vivo*. Potential early roles of IFN-t are unknown and deserve further studying. We speculate that signaling by the embryo modulates endometrial function to support embryo development.

A250 Embryology, Developmental Biology and Physiology of Reproduction

Estradiol modulates the expression of genes involved in the synthesis of endometrial PGF2a in cows

M.L. Oliveira[1], A.M. Gonella-Diaza[1], S.C. Scolari[1], R.S. Ramos[1], G. Pugliesi[1], M. Binelli[1], C.M.B. Membrive[2]

[1]USP, Pirassununga; [2]UNESP, Dracena.

Keywords: luteolysis, prostaglandin, endometrium.

In cattle, estradiol exerts an important role in endometrial PGF2α release, associated with luteolysis. The production of PGF2α results from the stimulation of oxytocin receptors and the subsequent activation of a cascade of intracellular events. . However, the molecular mechanisms by which estradiol acts in the endometrial PGF2a synthesis are little known.. The hypothesis is that estradiol stimulates gene expression of proteins involved in PGF2α synthesis in the bovine endometrium. Nelore (N = 52), pluriparous, cyclic and non-lactating cows received 2 mg of estradiol benzoate (Sincrodiol Gold fino® Cravinhos, Brazil) and an intravaginal progesterone device (1g; Sincrogest; Ourofino®, Cravinhos, Brazil) during 8 days. The cows received 0.5 mg of sodium cloprostenol (Sincrocio; Ourofino, Cravinhos, Brazil) via IM, 48 hours before the device removal and a second application at the removal. On day 15 of the estrous cycle (D0; estrus) the following treatments were administered: placebo (P; 5 ml of ethanol 50%; IV), estradiol (E; 5mL of 50% ethanol containing 3 mg of 17β estradiol; IV) or control (not treated). The time of treatments application was considered as time 0. Cows were subjected to a transcervical endometrial biopsy, and according to the time of biopsy were divided into the following groups: control group on time 0 (C; n = 10), time 4 hours (E4; P4 n = 11 and n = 10) or time 7 hours (E7, n = 10 and P7; n = 11). The tissue obtained by biopsy was analyzed by qRT-PCR for relative quantification of the genes : PTGS2, PLA2G4, ESR1, ESR2, OXTR, PKCα, PKCβ, AKR1CB1, and AKR1C4. The abundance of transcripts was analyzed by two-way ANOVA. The PTGS2 gene ($P < 0.05$) showed lower expression in P7 and E7 groups compared to P4 and E4 groups, respectively. Expression of PLA2G4 and ESR1 genes in the E4 group was lower compared to E7 ($P < 0.05$). There was lower expression of the gene ESR2 in E4 and E7 groups compared to the other groups ($P < 0.05$) and more OXTR transcripts in E4 and E7 groups ($P < 0.05$) compared to counterparts. There was a down regulation of PRKCα transcripts in the E7 group compared to other groups ($P < 0.05$). Also, a lower PRKCβ expression in P7 and E7 groups ($P < 0.05$) compared to the others. The expression AKR1B1 and AKR1C4 was lower ($P < 0.05$) in E4 and E7 groups compared to the other groups. It is concluded that, with the exception of OXTR, estradiol administration reduced gene expression of most of the proteins involved in PGF2a synthesis, indicating an acute negative feedback in the transcription of these genes. It is suggested that, at a time prior to the biopsies, there has been an increase in the expression of those genes. Thus, it becomes necessary to evaluate the protein expressioment.n, to determine if the decrease in the abundance of the transcripts is associated with an increase in the abundance of proteins after four hours of treat.

A251 Embryology, Developmental Biology and Physiology of Reproduction

Bovine embryo production under oxidative and glycemic-stress conditions is associated with disruption of transcriptional control

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

[1]USP, Sao Paulo; [2]UFABC, Santo Andre; [3]UNESP, Botucatu.

Keywords: hyperglicemia, oxidative stress, IVP.

Although Assisted Reproduction Techniques have been used for over 30 years, there are still concerns on how the manipulation of embryos may impact the health of future offspring. There are many forms of stress to which IVP embryos are exposed during in vitro culture. Numerous studies have shown that the exposure to a glucose environment and/or high oxygen tension may induce developmental arrest and poor quality blastocysts. The current challenge is to define markers of embryonic stress in order to formulate more appropriate culture conditions and improve the success rates of ART. In this context, the study of gene expression profile may be a valuable tool to assess stress-related impact and deviations from normal phenotype. Thus, this study aimed to gain insight as how the genome of embryos responds during IVC under different oxygen tensions and different glucose concentrations in culture media. For this purpose, bovine embryos were produced in vitro following conventional protocols in 4 replicates. Embryos were cultured in different oxygen tension (20 and 5%) from day 0 and also in different glucose concentrations (0.2, 0.6 and 1mM) after day 3. A total of 72 blastocysts from 6 groups were collected at day 7 and submitted to analysis. The transcription profile of 96 target genes was evaluated with BioMark HD System (Fluidigm, San Francisco, USA). After statistical analysis, genes were considered relevant when $1.5 \leq$ Fold Change ≤ 0.66. Although there is no statistical significance, blastocyst rates gradually increase with the increasing concentration of glucose (25.19% [0.2 mM], 29.92% [0.6 mM] and 36.09% [1 mM]) in 20% O_2 and (24.16% [0.2 mM], 26.11% [0.6 mM] and 37.35% [1 mM]) in 5% O_2. For relative transcripts quantification, the group 0.6 mM-5% O_2 was used as a physiological reference. A total of 56 genes presented differences in relative transcription in at least one group when compared to the reference. Moreover, 5 genes were found to be less abundant in all the other analyzed groups, while 7 genes were found to be over expressed when compared to the same reference. Deeper investigation revealed that embryos cultured either in high (1 mM) or low (0.2 mM) concentrations of glucose in both oxygen tensions presented an over expression of genes related to cell cycle (CDK6, NFKB2, PFKP), differentiation and quality (IFITM3, IGFBP4, PAF1, DDIT3). Besides, the transcription of genes related to apoptosis initiation (CASP9), mitochondrial function (AUH) and DNA repair (MORF4L2) is down regulated in the same groups. In conclusion, perturbations of developmentally important genes may translate into a stress response, affect embryonic quality and lead to abnormal phenotypes in the future livestock.

A252 Embryology, Developmental Biology and Physiology of Reproduction

Oocyte prematuration with forskolin and IBMX for two hours can modulate the lipid profile and gene expression of bovine blastocysts produced *in vitro*

E.M. Razza[1], M.J. Sudano[2], K.R.A. Belaz[3], P.K. Fontes[1], F.F. Franchi[1], P.H. Santos[1], M.N. Eberlin[3], M.F. Machado[1], M.F.G. Nogueira[4]

[1]Unesp-botucatu, Botucatu; [2]Unipampa, Uruguaiana; [3]UNICAMP, Campinas; [4]UNESP, Assis.

Keywords: prematuration, blastocyst, *in vitro*.

Different systems of in vitro maturation (IVM) have been extensively tested to improve in vitro production (IVP) efficiency. We tested a prematuration (PM) system that uses forskolin (FSK) and 3-isobutyl-1-methylxanthine (IBMX) in order to maintain optimum levels of cyclic adenosine monophosphate in the oocyte. However, the effects of this PM system on the blastocyst quality are still poorly understood. Lipid accumulation in IVP-embryos has been related to lower developmental potential and cryopreservation hability, especially when compared to embryos produced in vivo. The aim of our work is to evaluate if the PM of oocytes for 2 h with FSK and IBMX can differently affect the lipid profile and the mRNA transcript level of quality-related genes in blastocysts after IVP. Therefore, we submitted bovine cumulus-oocyte complexes (COC) to the PM system, in which COC were first cultured for 2 h in TCM199 media with FSK (100 μM) and IBMX (500 μM), followed by 24 h of conventional IVM [TCM199, recombinant human FSH (0.1 IU/mL), bovine serum albumine (4 mg/mL), pyruvate (0.011 g/mL), amikacin (16.67 mg/μL) – Pre-IVM group]. Simultaneously, COC were cultured for 24 h with the same IVM media described above for the conventional IVM and were used as a control group. Matured oocytes were equally fertilized and cultured to the blastocyst stage. The lipid profile of individual blastocysts was assessed by matrix assisted laser desorption Ionization – mass spectrometry (MALDI-MS). The mRNA abundance of 78 quality related genes was evaluated by the microfluidic dynamics array BioMark HD System™ (Fluidigm®). The MS data were acquired directly on single embryos, without extraction, in the positive ion mode using an Autoflex III MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) using 2,5-dihydroxybenzoic acid (DHB) matrix to favor the ionization of the lipids. Lipid data were analyzed by multivariate and univariate statistical approach with the MetaboAnalyst 3.0 software. The relative abundance of differentially expressed genes was calculated by ΔΔCt method (target gene in relation to the mean value of three housekeeping genes: GAPDH, ACTB and PPIA) and groups were compared via t-test with statistical significance when $P < 0.05$. Preliminary MALDI-MS results indicated strong variability of lipid ions of m/z 704, 727, 729, 733, 743, 745, 747, 749, 757, 759, 763, 775, 782, 785, 787, 789, 791, 815, 835, 837 and 860. The mRNA abundance of GPX4 and SREBP1 was higher in the control group when compared to Pre-IVM group. In addition, 4 genes tended to be down-regulated in the Pre-IVM group [IGFBP4, IFITM3, OCLN and PGK1 (0.05)].

A253 Embryology, Developmental Biology and Physiology of Reproduction

Gene expression in the corpus luteum following intrauterine pulses of low doses of prostaglandins E1 and F2alpha in cattle

J.C. Ochoa[1], G. Baez[2], L. Melo[3], J. Mota[1], F. Peñagaricano[4], A. Guerra[2], J. Pinheiro[1], R. Sartori[3], M. Wiltbank[2]

[1]UNESP, Botucatu; [2]University of Wisconsin, Madison; [3]USP, Piracicaba; [4]University of Florida, Gainesville.

Keywords: corpus luteum, luteolysis, prostaglandin E1.

In ruminants, natural luteolysis is characterized by the release of several pulses of prostaglandin F2alpha (PGF) produced by the uterus. PGF is the luteolytic hormone, whereas prostaglandin E1 (PGE1) is considered to be a luteoprotective mediator. In previous studies, low doses of PGF infused into the uterus at 6 hour intervals resulted in regression of the corpus luteum (CL). This study was designed to develop a model to study the effect of low doses of PGE1, also infused into the uterine lumen, on the luteal responses to intrauterine PGF. Cows on day 10 of the estrous cycle received intrauterine infusions of saline (0.1 ml of saline + 0.1 ml of DMSO), PGE (2mg of PGE1 in 0.1ml of DMSO) or PGF (0.25 mg of PGF in 0.1 ml of saline) at 6-h intervals in a 2 X 2 experimental design. Thus, there were four treatment groups: SALINE (4 saline infusions; n = 5), PGE (4 PGE1 infusions; n = 5), PGF (4 PGF infusions; n = 5), and PGE+PGF (4 PGE1+PGF infusions; n = 5). Radioimmunoassay was used to measure circulating P4 concentrations and luteal volume was determined by transrectal ultrasonography. A luteal biopsy was collected from each cow at 30 minutes after each infusion for later determination of gene expression in response to each treatment. The values were analyzed for differences between treatments using the Proc Mixed procedure of SAS and differences between means at specific timepoints were assessed using Fisher LSD. Concentrations of P4 in the PGF group decreased compared to those in the saline group by 12 h (48.9% of control) after first infusion of PGF, at 24 h (20.2% of control), and all subsequent time points ($P < 0.05$). No differences in circulating P4 concentrations were found between Saline, PGE, and PGF+PGE. There was also a decrease of luteal volume between the PGF group and the other three groups that was detectable at 24 (56.4% of control), 48 (30.6% of control), and 72 (20.4% of control) h after PGF treatment ($P < 0.05$). There were no differences in luteal volume between saline and PGE or PGE+PGF. Thus, simultaneous intrauterine infusion of a low dose of PGE1 blocked the luteolytic actions of intrauterine PGF pulses in cattle, as measured by changes in circulating P4 and luteal volume. Initial analyses of gene expression in the luteal biopsy taken after the third PGF pulse indicate a typical pattern of gene expression in response to the PGF treatments (FGF2, EGR1, FOS and FAS increased; PTGFR, VEGFA, NR5A1 and STAR decreased) and that simultaneous PGE1 treatment completely blocked these gene expression changes. Thus, intrauterine infusion of PGF and PGE1 seems to provide an excellent model for determining the patterns of gene expression involved in the luteoprotective effect of PGE1. This model should allow determination of the role of PGE1 in the mechanisms involved in rescue of the CL during early pregnancy.

A254 Embryology, Developmental Biology and Physiology of Reproduction

Evaluation of different strategies of mineral supplementation and vitamin injection (KIT Adaptador® MIN and Adaptador® VIT, Biogenesis Bagó) for improving pregnancy rates in beef cows

T. Santin[1], M. Maturana Filho[1], K.M. Lemes[1], M.A. Silva[1], R.L. Gonçalves[2], G.A. Mattioli[3], J.P. Mendes Lollato[2], J.M. Persico[2], E.C.C. Celeghini[1], E.H. Madureira[1]

[1]FMVZ-USP, Pirassununga; [2]Biogénesis-Bagó, Curitiba; [3]Universidad Nacional La Plata, La Plata.

Keywords: fertility, supplement, Doppler.

Strategic supplementation of vitamins and minerals during the pre-TAI period has been linked to improved reproductive performance in beef cows. Indeed, the lack of selenium, manganese, zinc and copper occurs widely in Brazil. These trace minerals are essential for antioxidative activities, which can contribute to improve fertility in beef cows. Injectable mineral supplementation is an economically viable option to meet the demand for these micronutrients, mainly in periods of higher needs. The aim of this study was to compare different strategies of mineral supplementation and vitamin injection (KIT Adaptador® MIN and Adaptador® VIT, Biogenesis Bagó) during the breeding season of Nelore cows (n = 408). The experiment was conducted in the beef cattle administrative sector of Pirassununga campus -USP , using a completely randomized design with factorial arrangement of treatments (2X2), and the factors assessed were mineral and vitamin supplementation. The experimental groups were: G1) two doses of KIT Adaptador®, 20 days apart ; G2) 1 dose of KIT Adaptador® at the beginning of the protocol; G3) 1 dose KIT Adaptador® 20 days before protocol and G4) control (placebo). Thus, animals (n = 408) were randomly divided among groups. The employed hormonal protocol was: D0 = Cronipres® Mono Dose implant insertion with 1 g P4 + application of 2 mg EB (Bioestrogen®, Biogenesis Bagó, Brazil); D8,5 = withdrawal of intravaginal P4implant + application of 300 IU eCG (Ecegon®, Biogenesis Bagó, Brazil), + 75 ug D-cloprostenol (PGF2a, Croniben®, Biogenesis Bagó, Brazil) + 1mg EB (Bioestrogen® , Biogenesis Bagó, Brazil). On D10 TAI was carried out during the morning. Cyclicity rate and pregnancy rate (PR) were evaluated by ultrasound (Mindray M5 Vet, with linear probe of 5.0 MHz). Diagnose of pregnancy was held 30 and 60 days after TAI. Data were submitted to analysis of frequency by PROC FREQ and to logistic regression analysis by PROC LOGISTIC, using the Statistical Analysis System (SAS, 9.3), adopting a significance level of 5%. There was an improvement on cyclicity ($P < 0.05$) of cows treated with KIT Adaptador® before the beginning of TAI protocol (G1 = 59.2%, G2 = 54.5%, G3 = 62.4% and G4 = 48.5%). Pregnancy rate at 30 days diagnose was higher for cows that received KIT Adaptador® (G1 = 52.5%, G2 = 61%, G3 = 57.7% e G4 = 51.5%). Pregnancy rate at 60 days ($P < 0.05$) was also higher in the treated animals (G1 = 51.4%, G2 = 60%, G3 = 55.7% and G4 = 49.5%). Moreover, there also was a greater cyclical rate ($P < 0.05$) in cows treated with Adaptador® kit, that were evaluated for resynchronization at 30 days diagnosis (G1 = 74.5% G2 = 64.3% G3 = 63.3% and G4 = 55.4%). Cows receiving the KIT Adaptador® supplementation had higher mean follicular diameter at the time of insemination (G1 = 15.9% G2 = 15.4%, G3 = 14.8% G4 = 13.1%) and a greater vascularization (G1 = 61.4%, G2 = 59.6%, G3 = 62.1% e G4 = 451.4 %) of the largest follicle evaluated at TAI ($P < 0.05$). Therefore, the strategic supplementation with KIT Adaptador® MIN and Adaptador® VIT (Biogenesis Bagó) 20 days before or at the beginning of the protocol was efficient and economically viable for improving results of TAI protocols in beef cattle. The two doses were effective for improving results of the first TAI protocol, as well as for the maintenance of cyclicity until the resynchronization program.

A255 Embryology, Developmental Biology and Physiology of Reproduction

Caspase15 gene expression in swine embryos of different genetic groups

L.S. Amorim[1], T.S. Kawamoto[2], S.E.F. Guimarães[3], P.V. Silva[3], P.S. Lopes[3], J.D. Guimaraes[4]

[1]BIOTRAN, Cruz das Almas; [2]UFU, Uberlandia; [3]Departamento de Zootecnia Universidade Federal de Viçosa, Viçosa; [4]Departamento de Veterinária Universidade Federal de Viçosa, Viçosa.

Keywords: apoptosis, transcripts, pig.

Apoptosis has an important role in pre-implantation embryo development because embryonic cells are subjected to DNA damage that can activate repair mechanisms or induce apoptosis. The latter culminates in the activation of the caspase enzyme responsible for cleaving various cellular substrates. The aim of this study was to measure differences in Casp15 gene expression between Piau embryos and embryos from a swine commercial line. Gilts were slaughtered or subjected to exploratory laparotomy for recovery of embryos on day 6 after mating (day 0 = estrus). Surgery was performed as previously described (RD Cameron 1989 Australian Veterinary Journal, 66, 314-318) and approved by the Ethics Committee of the UFV. Commercial line embryos were obtained by flushing the uterine tract post mortem 6 days post mating, using PBS at 37 ° C plus 1% PVA. Embryos were classified according to the recommendations of the IETS (International Embryo Transfer Society, 1998) and 65 grade I blastocysts were selected for the study (35 and 30 to Piau and commercial line, respectively). The embryos were preserved in 2 mL of PBS with 1% PVA in cryotubes, and kept in liquid nitrogen until RNA extraction. The extraction was performed using RNeasy Mini Kit (Qiagem, Valencia, CA). Total RNA was reverse transcribed with M-MuLV ProtoScript First Strand cDNA Synthesis kit (Biolabs, Ipswich, MA). Real-time polymerase chain reaction was performed using GoTaq qPCR Master Mix (Promega, Madison, WI) in an ABI PRISM 7300 Sequence Detection Systems (Applied Biosystems, Foster City, CA). Primers were designed from pig sequences available in GenBank. The linearity of Casp15 mRNA amplification was similar to reference gene (GAPDH). The reactions were performed using 200 nM and 400 nM primers for Casp15 and GAPDH, respectively, and 1.6 ng of cDNA reaction for each gene. The thermocycling conditions consisted of 40 cycles of 30 sec melting at 95 °C followed by 30 seconds annealing and extending at 60 °C. After amplification, an analysis of the melting curve was performed to confirm the absence of unspecific products . Gene expression data were evaluated using the 2-ΔCt method (KJ Livak and Schmittgen TD 2001, Methods, 25, 402-408). The results of gene expression were analyzed using the GLM procedure (SAS Institute, Cary, NC). The Casp15 mRNA expression did not differ between the preimplantation embryos from Piau and commercial line ($P > 0.05$). Despite the fact that no difference was found, the development of Piau embryo and litter size are different compared to other races.

A256 Embryology, Developmental Biology and Physiology of Reproduction

Prevalence of Brucellosis, Leptospirosis and Neosporosis in cows with retained placenta in southwest of Parana, Brazil

Y.F. Porto[1], A. Pinto-Neto[1], F. Bernardi[2], M.G. Possa[2], G. Vendruscolo[1], M.F. Mota[1], A.C. Martinez[3], R.C. Berber[4]

[1]Universidade Federal da Fronteira Sul - UFFS, Realeza; [2]Autonomo, Realeza; [3]Universidade Estadual de Maringá, Umuarama; [4]Universidade Federal do Mato Grosso, Sinop.

Keywords: dairy cattle, reproductive diseases, reproductive efficiency.

The aim of this study was to measure the prevalence of Brucellosis, Leptospirosis and Neosporosis in cows with retained placenta in the southwest of Parana, Brazil. Eighty-four dairy cows diagnosed with placenta retention (PR: partial or total presence of fetal membranes in the uterus after 12 hours post partum). Animals were diagnosed using: sorological test of screening Buffered Acidified Antigen for Brucelosis; microscopic agglutination test for Leptospirosis; and indirect immunofluorescence test for Neosporosis. Animals were considered positive when presenting title > 1:100 (Leptospirosis) and 1:200 (Neosporosis). None of the animals with PR was detected with Brucella abortus bacteria; nevertheless 39% (33/84) were reactive to one or more serovars of Leptospira, 15% (13/84) were positive to Neospora caninum, and 4% (4/84) were reactive for Leptospirosis and Neosporosis. In conclusion, data of present study gatter attention to leptospirosis and neosporosis as associated with PR in dairy cows of southwestern Parana.

A257 Embryology, Developmental Biology and Physiology of Reproduction

Effect of mineral supplementation and vitamin injection (Kit Adaptador® MIN and Adaptador® VIT, Biogenesis Bagó), associated with vaccination against reproductive diseases (Bioleptogen® and Bioabortogen® H, Biogenesis Bagó) on pregnancy rates in beef cows

M.A. Silva, T. Santin, M. Maturana Filho, K.M. Lemes, R.L. Gonçalves, G.A. Mattioli, J.P. Mendes Lollato, J.M. Persico, E.C.C. Celeghini, E.H. Madureira

FMVZ-USP, Pirassununga.

Keywords: fertility, injectable supplementation, reproductive vaccines, Nelore cows.

Injectable supplementation of vitamins and minerals during the period of greatest activity and stress can improve antioxidative response, mainly by meeting the demand for micronutrients such as Selenium, Manganese, Zinc and Copper and vitamins such as Retinol (A) and Tocopherol (E), which are deficient in pasture in most of the Brazilian territory. These trace minerals are essential for antioxidative activities; therefore, the strategic supplementation of these elements, in periods as management during the breeding season and the vaccination period, can contribute to improve fertility in beef cows. Injectable mineral supplementation is an economically viable option to meet the demand for these micronutrients, mainly on higher demand periods. Reproductive diseases significantly affect the reproductive performance of cattle in Brazil. The aim of this study was to verify the efficiency of mineral supplementation and vitamin injection (Kit Adaptador® MIN and Adaptador® VIT, Biogenesis Bagó), associated with vaccination against leptospirosis and IBR / BVD (Bioleptogen® and Bioabortogen® H, Biogenesis Bagó) during the breeding season in improving the fertility of Nelore cows (n = 532). The experiment was conducted in the beef cattle administrative sector of campus of USP in Pirassununga, using a completely randomized design with factorial arrangement of treatments (2X2), and the factors assessed were mineral and vitamin supplementation (KIT Adaptador®, Biogenesis Bagó) and vaccination against leptospirosis and IBR / BVD, in a total of four groups. Thus, animals (n = 532) were randomly divided between groups. Treated animals received the first dose of KIT Adaptador® and also a vaccine against reproductive IBR/BVD and leptospirosis 20 days before the beginning of the TAI protocol. On day 0 of reproductive protocol the second dose of treatment and vaccine were administered. The used hormonal protocol was: D0 = Cronipres® Mono Dose implant insertion with 1 g of P4 + application of 2mg of EB (Bioestrogen®, Biogenesis Tobago, Brazil); D8,5 = withdrawal of intravaginal implant of P4 + application of 300 IU of eCG (Ecegon®, Biogenesis Bagó, Brazil), + 75ug of D-cloprostenol (PGF2a, Croniben®, Biogenesis Bagó, Brazil) + 1mg BE (Bioestrogen® , Biogenesis Bagó, Brazil). On D10 TAI was made during the morning. Cyclicity rate and pregnancy rate (PR) were evaluated by ultrasound (Mindray DP2200 Vet with linear probe of 5.0 MHz). Diagnose of pregnancy was held 30 and 60 days after TAI. Data were subjected to analysis of frequency by PROC FREQ and to logistic regression analysis by PROC LOGISTIC, using the Statistical Analysis System (SAS, 9.3), adopting a significance level of 5%. There was an improvement on cyclicity ($P < 0.05$) of the animals receiving KIT Adaptador® (C = 41.7%; Vac = 60.8%; Adapt = 67.7%; Adapt + Vac = 54.3%) and there was an interaction between treatments (P = 0.02 Adapt; P = 0:05 interaction) in pregnancy rate at 30 days (C = 48.9%; Vac = 55.4; ADAPT = 70.8; Adapt + Vac = 64.1), and at 60 days ($P < 0.05$) there only was a KIT Adaptador® effect (C = 41.7%; Vac = 52.1%; Adapt = 63.7%; Adapt + Vac = 55.4%). As a conclusion, the strategic supplementation with KIT Adaptador® MIN and Adaptador® VIT, Biogenesis Bagó, associated to vaccines for reproductive diseases is an efficient and cost-effective strategy for improving results in TAI programs for beef cattle.